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Constituents in Cascara Sagrada Extract

IV. Aloe-Emodin, Chrysophanic Acid and Emodin*

By Robert W. Liddell, C. G. King and George D. Beal†

A number of publications have dealt with the isolation and biological assay of individual compounds that occur in, or can be derived directly from, extracts of the dried bark from *Rhamnus Purshiana* trees (cascara sagrada). The isolated compounds to which cathartic activity has been attributed are all hydroxy-methylanthraquinones of the emodin type. A large part of the anthraquinone material is apparently linked in glycosidic form to rhamnose and glucose, the two sugars being present in approximately equal quantities. In no case, however, has an isolated compound been found to exert a physiological activity that was comparable with the activity of the standard U. S. P. crude extract (on the basis of the extract's content of known compounds).

Emodin was identified in the drug by the early studies of Schwabe (1) and Jowett (2). The latter also identified isoemodin, rhamnol, syringic acid, pyrocatechuic acid and two of the fatty acids. Sipple, King and Beal (3) isolated frangulin, the rhamnoside of emodin, and Green, King and Beal (4) reported the identification of isoemodin and methylhydrocotoin. Daels (5) and Beal and Tumminkatti (6) showed that the emodin-type constituents were characteristically present in both the free form and as glycosides. Proof of the structure of emodin as 1,6,8-trihydroxy-3-methylanthraquinone was established by the studies of Eder and Widmer (7) and Jacobson and Adams (8). Gunton and Beal (9) also synthesized the natural rhamnoside of emodin, frangulin.

The present study was directed toward (*a*) further isolation of active compounds and also toward (*b*) checking the activity of known mixtures of pure compounds to test their synergistic effects in comparison with single compounds and the crude extract. We believe that the latter part of the study has provided an essentially new experimental basis for interpreting the activity of cascara sagrada and perhaps of many other crude extracts of a similar nature.

EXPERIMENTAL

A preliminary concentration of the most active material in cascara sagrada was accomplished as follows. The standard U. S. P. fluidextract of cascara sagrada (100 ml.) was dialyzed in cellophane tubes against 10 volumes of distilled, deaerated water for 48 hrs. Carbon dioxide was passed in during this time to exclude oxygen, pro-

* Contribution No. 456 from the Department of Chemistry, University of Pittsburgh. The authors are indebted to Parke, Davis and Company for a research grant in support of the present investigation.

† Assistant Director, Mellon Institute of Industrial Research, Pittsburgh, Pa.

mote stirring and prevent mold growth. Nearly pure emodin occasionally separated from the dialysate, apparently subsequent to hydrolysis of a glycoside or other soluble conjugate. The dialysate was concentrated to 50 ml. *in vacuo* and then hydrolyzed by refluxing in the presence of 250 ml. of chloroform at *p*H 1.0 for 2½ hrs. After cooling and separation of the two layers, it was found that practically all of the activity was in the aqueous portion. This layer was brought to the *p*H of the original extract (4 to 5) by treatment with sodium bicarbonate, and then treated with 7 volumes of acetone or absolute methyl alcohol to remove inorganic and tarry organic material. The results with acetone were more variable, so methyl alcohol was used in the later work. The insoluble material was centrifuged and discarded as it was found to be almost inactive. The dilute methyl alcohol solution was then evaporated to dryness *in vacuo* at approximately 40° C., using a capillary flow of carbon dioxide. The spongy, dried material was extracted by stirring with 25 ml. and then 15 ml. of absolute methyl alcohol. About 50 ml. of water was then added to the solution, and the methyl alcohol and part of the water were removed *in vacuo*. This water-soluble extract contained approximately 7 Gm. of solid material and had from two to three times the biological activity of the fluidextract on a total solids basis. The solids were relatively insoluble in ethyl acetate, chloroform and butanol. At room temperature in aqueous or methanol solutions, there was a fairly marked loss in activity after storage for only a few days.

Isolation of Aloe-Emodin.—An aqueous solution of 10 Gm. of the methyl alcohol-soluble material was treated with ferric chloride solution according to the method of Cahn and Simonsen (10). Because extraction of the resulting tar with toluene or other organic solvents gave only a small yield of anthraquinones, it was found necessary to dissolve the tarry material in 5% sodium hydroxide solution. The solution was then acidified and stirred with several volumes of ether. After centrifuging to separate the ether and water layers, the ether solution was decanted. The solid material in the aqueous layer was again dissolved in dilute sodium hydroxide solution, and the acidification and ether extraction were repeated. From four to seven such treatments were necessary for the removal of the anthraquinones. The ether solution was then extracted with small portions of 5% sodium hydroxide solution until the extracts were almost colorless. The aqueous solution was in turn acidified and extracted with ether until the ether extracts were almost colorless. The entire ether solution was then extracted five times with 50-ml. portions of 5% sodium bicarbonate solution. This treatment removed a large amount of the color, but left the anthraquinone content practically unchanged. The ether solution was then extracted twice with 50-ml. portions of 5% sodium carbonate to remove small amounts of β-hydroxyanthraquinones (with an intervening water wash), washed several times with water and then evaporated to dryness. The residue was extracted with small amounts of boiling toluene until almost completely dissolved. On cooling, aloe-emodin crystallized from solution. After recrystallization from toluene and glacial acetic acid, it formed orange-red shiny platelets or needles which melted at 218–219° C. Further purification by sublimation *in vacuo* brought the melting point to 222–223° C. Yield, 4% to 8%. Analysis: Calcd.: C, 66.64%; H, 3.73%. Found: C, 66.62% and 66.42%; H, 3.81% and 3.86%. The crystals were very soluble in pyridine, easily soluble in glacial acetic acid, soluble in acetone, slightly soluble in ethyl alcohol, methyl alcohol, ether, chloroform, benzene and toluene, and insoluble in water and petroleum ether.

Upon treating aloe-emodin with acetic anhydride and sodium acetate, the triacetate was obtained as golden needles (from ethyl alcohol) which melted at 175° C. Heating the aloe-emodin in a sealed Pyrex tube for 2 hrs. in a boiling water bath with benzoyl chloride and pyridine, followed by triturating with water and 5% sodium hydroxide solution, drying the residue and crystallizing from benzene, gave the benzoate as pale green needles which melted at 234–235° C. Refluxing the aloe-emodin for 1½ hrs. with a 1:1 mixture of hydriodic acid (sp. gr. 1.7) and glacial acetic acid, or reduction with tin and hydrochloric acid, formed an anthrone which crystallized from ethyl alcohol in pale orange platelets, melting point 203–204° C. The anthrone yielded an acetyl derivative which melted at 238–239° C. with darkening. All of the above derivatives that were prepared from aloe-emodin corresponded in properties with the derivatives recorded in the literature.

Isolation of Chrysophanic Acid.—The toluene filtrate, after separation of the aloe-emodin, was evaporated and treated with 25 ml. of chloroform. The mixture was allowed to stand over night, and any precipitate that formed was removed by centrifuging. The solution was then extracted a number of times with 0.5% potassium hydroxide solution to remove any remaining aloe-emodin and with 5% sodium hydroxide solution to dissolve the chrysophanic acid. Carbon dioxide was passed into the solution until a heavy brown precipitate was formed. The precipitate was washed several times with 5% sodium carbonate solution and water, dried by evaporation at room temperature and recrystallized from acetic acid, petroleum ether and ethyl alcohol, giving orange-yellow needles melting at 190–191° C. No depression of melting point resulted when the crystals were mixed with chrysophanic acid (m. p. 191–192° C.) prepared from chrysarobin by the method of Gardner (11). Yield, 0.5% to 1.5% on a dry solids basis.

Chrysophanic acid was nearly insoluble in dilute sodium carbonate solution but dissolved slowly with an intense red coloration in dilute sodium hydroxide. It was somewhat more soluble than aloe-

emodin in toluene and much more soluble in chloroform. By use of the methods referred to for aloe-emodin, the material formed an acetyl derivative, pale yellow in color, melting at 205–206° C. The benzoyl derivative was prepared and crystallized from acetic acid diluted with hot ethyl alcohol, as pale yellow prisms melting at 202–204° C. Reduction with hydriodic acid or by the method of Gardner, using tin and hydrochloric acid, gave an anthrone melting at 202–203° C. The acetyl derivative of the anthrone melted at 237–238° C. Refluxing with constant-boiling (48%) hydrobromic acid and acetic acid, or treatment with 80% sulfuric acid at 160° C. for 20 min., produced no change in the melting point, showing that the compound was not a methyl ether.

The melting point of the product corresponded with that of the chrysophanic acid obtained from rhubarb by Tutin and Clewer (12), and from frangula by Gunton and Beal (9), but this is lower than the value given for chrysophanic acid that was prepared synthetically (193–194° C.). The color of the crystalline material was more orange than that of the synthetic material, or of that prepared from chrysarobin. However, all of the derivatives that were prepared gave the correct melting points.

Isolation of Syringic Acid.—The soluble material in the standard aqueous solution (equivalent to 200 ml. of fluidextract) was treated with 50 ml. of concentrated hydrochloric acid and then hydrolyzed in a Pyrex bottle for 6 hrs. at 125° C. in the autoclave. Both the acid solution and the tar were extracted several times with 30-ml. portions of ether. The solvent was allowed to evaporate and the brown, semi-crystalline material was dissolved in acetone, treated with Norit and the acetone allowed to evaporate. After recrystallization from glacial acetic acid and then from water, the product formed long colorless needles melting at 205–206° C. yield, 100–200 mg.

Acetylation with acetic anhydride and sodium acetate, followed by crystallization from dilute acetic acid or ethyl alcohol, gave an acetate which melted at 190–191° C. Syringic acid was also obtained by hydrolysis of the standard extract in 0.5 *N* sodium hydroxide solution.

Repeated attempts (over a wide *p*H range and with many different reducing agents) to concentrate and then purify the anthraquinone types of material in the fluidextract by the procedure of reduction to form products with a higher solubility in water, followed by aeration or chemical oxidation to re-form the less soluble quinones, did not give encouraging results. The precipitations secured by oxidation were slight, and the products could not be purified with as good success as that achieved by the procedures described above.[1]

Bioassays.—Although the anthraquinones obtained from cascara by degradation processes do not in all cases possess the same structure as when present in the fluidextract, particularly with regard to glycosidic linkage, it was thought desirable to assay them in order to relate structure to activity and to test the synergistic activity of known mixtures in comparison with single components. An earlier study had shown that hydrolysis of the native glycosides exerted little effect upon their cathartic activity, although oxidation may cause fairly steady losses in potency (4). The emodin and aloe-emodin were obtained from cascara. The chrysophanic acid was prepared from chrysarobin by the method of Gardner, since the low yields that could be obtained from cascara made that source less satisfactory. The solid products were fed in capsules; the capsules were "washed down" with 1 to 2 ml. of water or dilute sucrose solution.

When fed individually in 25-mg. dosage, the three compounds showed negligible or only slight cathartic activity. Mixtures of 10 mg. of emodin with 10 mg. of chrysophanic acid, 10 mg. of aloe-emodin with 10 mg. of emodin, and 10 mg. of aloe-emodin with 5 mg. of chrysophanic acid were found to be only slightly to moderately active. These results were in striking contrast, however, with the subsequent tests in which mixtures of all three compounds were fed. A 25-mg. (total) dosage of the tripartite mixture was highly active. Even one-half of this amount of the mixture (12.5 mg.) showed moderate activity. For example, a mixture of 10 mg. of aloe-emodin, 10 mg. of emodin and 5 mg. of chrysophanic acid possessed a very strong cathartic activity; and one-half of this dosage showed greater activity than 25 mg. of any one of the individual substances.

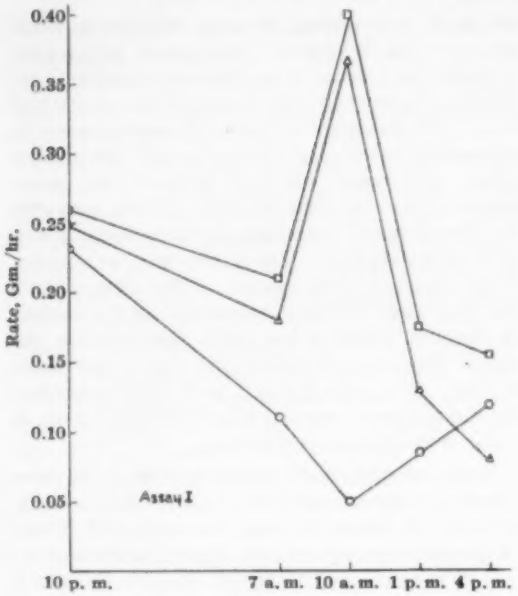
Chrysophanic acid anthrone was inactive in 25-mg. dosage, aloin showed only slight activity in 25-mg. dosage, and chrysarobin in the same dosage showed only slight or no activity.

After the anthraquinones were fed, they could be detected in the feces by treatment with dilute alkali solutions. The urine from treated animals was also reddened when made alkaline with sodium or potassium hydroxide solution; the coloration effect was particularly strong with emodin, less evident with aloe-emodin and weak with chrysophanic acid.

Groups of 15 to 20 animals (preferably with an average weight of approximately 300 Gm.) for each test level gave reasonably consistent results. A preliminary 6-hr. record of the fecal output rate served both to eliminate animals with abnormal records and to match the satisfactory animals, so that their group averages were comparable at the beginning of the assay. A reasonably convenient schedule was obtained by feeding the test preparation at 10 p. m., followed by collections for weighing at 7 a. m., 10 a. m., 1 p. m. and 4 p. m.

The activity of fluidextracts obtained from different sources varied considerably, although the total solids value was about 0.25 Gm. per ml. in each case. Fluidextracts prepared in the laboratory according to the directions given in the U. S. P.

[1] The authors are indebted to Mr. Charles Spiegl for his assistance in carrying out a number of chemical studies and for making a recheck of the assay values reported in the present paper.



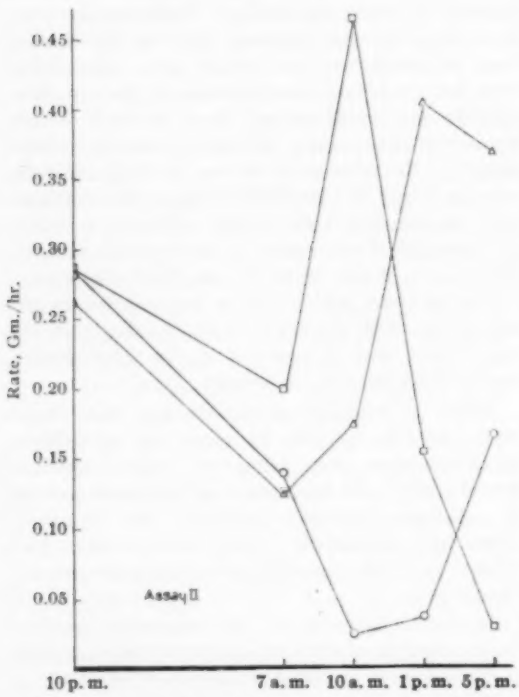
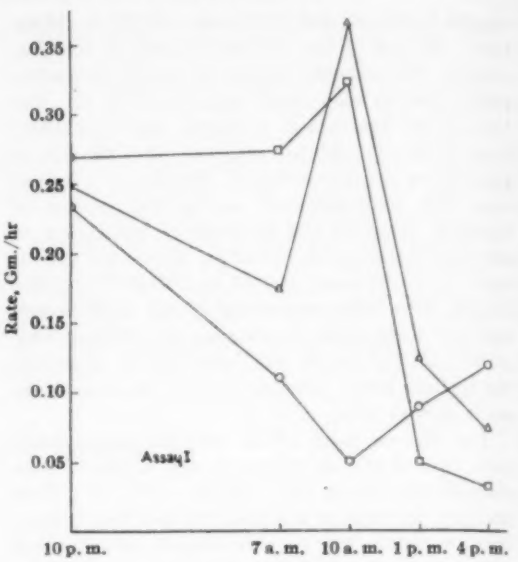


Fig. 1—Assay of mixture containing emodin (10 mg.), aloe-emodin (10 mg.) and chrysophanic acid (5 mg.). Twenty guinea pigs in each test group. The 1.5 ml. of cascara sagrada extract contained approximately 250 mg. total solids.

□ Aloe-emodin (10 mg.)
Emodin (10 mg.)
Chrysophanic acid (5 mg.)
Δ Cascara (1.5 ml.)
○ Negative controls

XI were found to be uniformly active in 1-ml. dosages, but 1.5-ml. portions of the four commercial samples tested were required for a comparable effect.



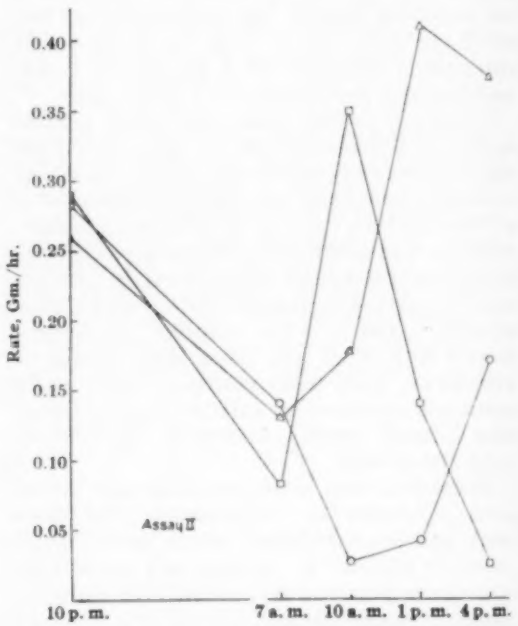


Fig. 2—Assay of mixture containing emodin (5 mg.), aloe-emodin (5 mg.) and chrysophanic acid (2.5 mg.). Twenty guinea pigs in each test group.

□ Aloe-emodin (5 mg.)
Emodin (5 mg.)
Chrysophanic acid (2.5 mg.)
Δ Cascara extract (1.5 ml.)
○ Negative controls.

DISCUSSION

In all, considerable information has been gained concerning the active ingredient of cascara sagrada. The fact that the active materials are water-soluble and will dialyze rapidly through a viscose membrane indicates that the most important compounds are of relatively low molecular weight. However, the products are not very stable; they tend to be oxi-

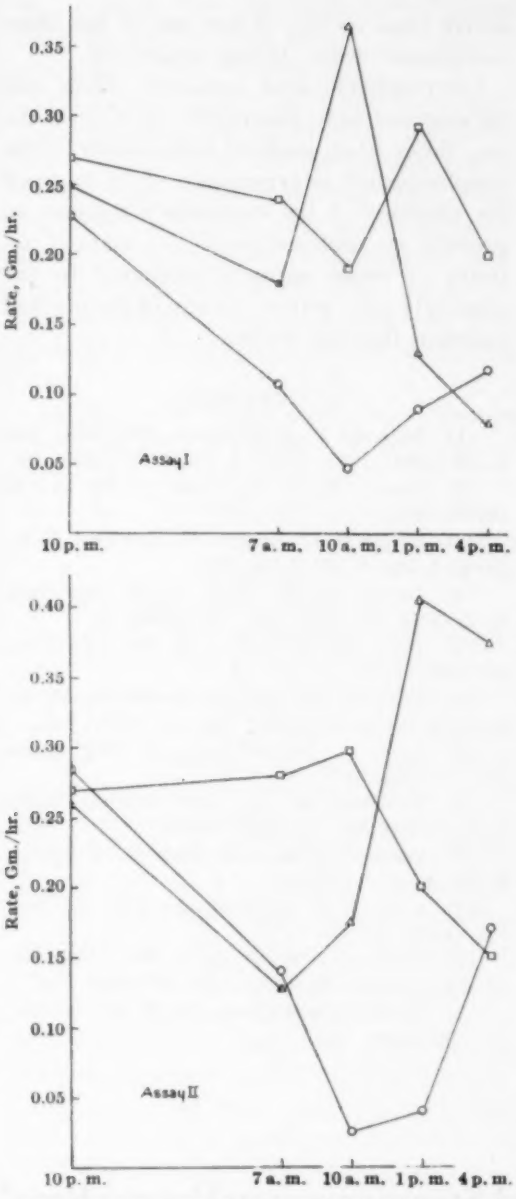


Fig. 3.—Assay of mixture containing aloe-emodin (15 mg.) and chrysophanic acid (10 mg.). Eighteen guinea pigs used in each test group.

□ Aloe-emodin (15 mg.)
Chrysophanic acid (10 mg.)
Δ Cascara extract (1.5 ml.)
O Negative controls.

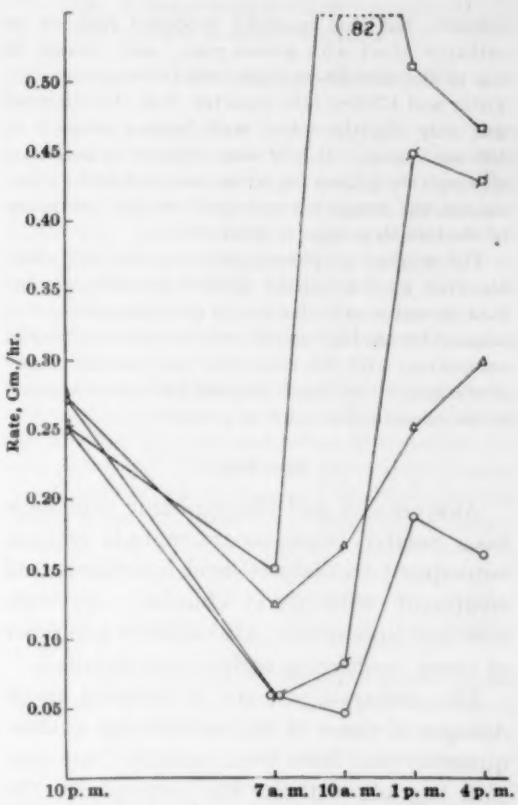


Fig. 4.—Assay of aloe-emodin and mixture of compounds obtained from cascara sagrada extract. Twelve guinea pigs in each test group. The mixture had the same composition as that referred to in Figs. 1 and 2.

Δ Aloe-emodin (25 mg.)
□ Mixture (25 mg.)
ø Mixture (12.5 mg.)
O Negative controls.

dized in parallel with condensation or polymerization reactions during further treatment, making the isolation of a high percentage of the initial individual compounds present in crude extracts exceedingly difficult. Some substances in the active fraction are apparently closely related to aloin, since they (*a*) give (initially), in borax solution, a strong fluorescence with ultraviolet light, (*b*) are difficult to hydrolyze and (*c*) yield hydroxy-anthraquinones after treatment with ferric chloride.

The fact that the Bornträger reaction for anthraquinones was strongly positive in the urine and feces after administration of the compounds assayed gives an indication that these materials were in large part excreted unchanged; at least they did not undergo simple reduction to the anthrone series of products, since they gave no borax fluorescence test in ultraviolet light. Further destruction of the compounds that undergo reduction to the anthrone stage appears probable, since after feeding chrysophanic acid anthrone, no test for either the anthrone or the corresponding anthraquinone could be obtained in the urine or feces.

The method of bioassay with guinea pigs gave occasional erratic results, but repeated tests with animals that were comparable in size and age were in reasonably good agreement, even when carried out by two independent investigators at different times of the year. The results did not fully conform with those recorded for humans, however. In the latter case, very few definite records are available. Thus it has been reported that 25 mg. of aloin is a cathartic dose for most human

subjects, but that quantity produced little or no cathartic effect with guinea pigs; and, though 50 mg. of aloe-emodin was quite active for guinea pigs, Tutin and Clewer (12) reported that this material was only slightly active with human subjects in 100-mg. dosage. It is of some interest to note that although the guinea pig serves as a satisfactory test animal, the dosage per unit body weight, compared to the human dosage, is about 250:1.

The marked supplementary or synergistic effect observed when a mixture of three products was fed is of special interest because it provides evidence to account for the high activity of the crude extracts in comparison with the relatively low activity of any of the isolated or highly purified fractions contained in the conventional types of extracts.

SUMMARY

Aloe-emodin and chrysophanic acid have been isolated from cascara sagrada extract, subsequent to dialysis, acid hydrolysis and treatment with ferric chloride. Syringic acid and emodin were also isolated a number of times, confirming earlier identifications.

The cathartic activity of weighed single dosages of three of the substituted anthraquinones that have been isolated from cascara sagrada extract was assayed by the technique previously described, using guinea pigs as test animals. Known, weighed mixtures of the three ingredients were also assayed and found to be markedly more active than similar quantities of any one of the compounds given alone. A mixture of 10 mg. each of aloe-emodin and emodin plus 5 mg. of chrysophanic acid was distinctly more active than 25 mg. of any one of the three compounds when tested separately.

Chrysophanic acid anthrone, aloin and chrysarobin were practically inactive in 25-mg. doses when assayed individually. The supplementary or synergistic effect observed for mixtures of the compounds appears to provide an explanation of the marked activity of crude extracts compared to the relatively poor activity of any of the purified fractions thus far studied.

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A Study of Wild and Cultivated Stramonium in Puerto Rico*

By Carl H. Johnson† and Esteban Nunez-Melendez‡

Since stramonium grows profusely in Puerto Rico and since the leaves are widely used in the island for the relief of asthma and other ailments, a study of the content of the active medicinal principles of Puerto Rican stramonium was deemed desirable. A comparison of the wild and cultivated forms was made, together with the effects of fertilizer.

The three species of the genus Datura in Puerto Rico according to Britton (1) are *Datura Stramonium* L., *Datura Metel* L. and *Datura fastuosa* L. Their morphology has been studied by de Grosourdy (2), Stahl

* This paper is based on a thesis presented to the Graduate Council of the University of Florida in partial fulfillment of the requirements for the degree of Master of Science in Pharmacy.

Presented to the Scientific Section of the A. PH. A., Detroit meeting, 1941.

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‡ Instructor in Pharmacy, College of Pharmacy, University of Puerto Rico.

(3), Britton (1) and other investigators. Only the first species, *Datura Stramonium*, was used in this investigation.

The common name used in Puerto Rico, *belladonna del pobre*, signifies that the poorer classes cannot afford to buy preparations of the imported belladonna, but instead use the wild stramonium that grows so abundantly. For the relief of asthma the sun-dried leaves are made into cigarettes; when mixed with lard as an ointment they are used as an antispasmodic and for healing sores.

In 1936 stramonium seeds were obtained from the United States Department of Agriculture and were planted in the Medicinal Plant Garden of the College of Pharmacy of the University of Puerto Rico, with the purpose of comparing the plants of the wild and cultivated stramonium.

It is generally known that stramonium is abundant on the island, but the wild drug is frequently of inferior quality and below the strength of the United States Pharmacopœia (4). Among the factors that might affect the plant in the production of its active constituents are fertilization, soil, temperature, selection and other conditions. A study of the literature on stramonium reveals that there are many conflicting opinions regarding the various factors which affect the alkaloidal content of this plant.

That stramonium grown in different countries varies in the percentage of active constituents has been shown by many investigators. It has been demonstrated that leaves from South Africa contain over 0.5% of alkaloids (5), whereas leaves from China contain less than 0.2% (6).

EXPERIMENTAL

FIELD WORK

In view of the objectives of the present investigation, it was believed advisable to make an outline for the work on fertilization and on the cultivation of the seeds. Therefore the work was arranged as follows:

1. Study of the wild plants.
2. Study of cultivated plants from seeds obtained from wild plants.
   (a) Without using fertilizer.
   (b) Using a commercial fertilizer.
3. Study of cultivated plants from seeds obtained from the Department of Agriculture.
   (a) Without using fertilizer.
   (b) Using a commercial fertilizer.

From the wild plants the seeds were collected in September.

The seeds were planted in early spring in large seed beds about 3 ft. wide and 5 ft. long. The beds were carefully prepared by thoroughly mixing equal parts of rich garden soil, well-rotted manure and clean sand. After leveling off the surface the seeds were planted in drills about 1 in. deep and 4 in. apart. The seeds were barely covered with soil. Watering was done with a very fine spray to maintain the proper moisture in the soil, especially during dry weather.

Germination began quickly, the plants appearing above the ground in one week. The percentage of germination was high and plants of a uniform size were produced. The seedlings were allowed to grow in the beds until they attained a height of about six inches when they were strong enough to withstand transplanting. The seedlings were planted about 20 in. apart in the row with two rows about 3 ft. apart in each plot. It was observed that the seedlings developed rapidly into quite strong and healthy plants.

For the preparation of the plots, a new ground plow was used after first clearing off the brush. The plowing was repeated three times at one-week intervals. Because of good weather conditions the soil was well pulverized; in addition all the weeds were destroyed and the soil was aerated. Practically no clods remained and the soil was in good condition for putting out the plants. The field was divided into plots about 40 ft. long and 5 ft. wide, separated by furrows 1 ft. wide and deep. Six plots were established for each group of plants.

The plants grew very well and proper care was exercised to keep the soil free from weeds at all times. The soil was cultivated with a hoe about twice a month, particularly after each hard rain. In hot, dry weather the cultivation was shallow to conserve the natural moisture of the soil. Water was used when necessary.

The commercial fertilizer used contained 4% nitrogen, 8% phosphorus and 4% potash.

The leaves were harvested at the beginning of the month of July. At this time the plants were in full bloom and appeared to be at the height of their development. There was no appreciable difference in the size of the leaves of plants grown under different conditions nor did the plants themselves give any indication of the soil variations.

LABORATORY WORK

The harvested leaves were air dried at a temperature of about 40° C. by spreading them out in a thin layer in an attic. As the weather was dry and warm, no artificial heat was necessary. After about one week's time, the leaves were ground in a hand drug mill until all of the drug could pass through a 60-mesh sieve. After the material was sifted, it was placed in a tightly stoppered, amber-colored con-

tainer which was kept away from the light in a cool, dry place.

In addition to the assay for alkaloidal content of the plant, other determinations were carried out which might give some valuable information as to the influence of fertilization and climatic factors. Therefore, the following assays were carried out on each sample of the drug cultivated under the different conditions set forth:

1. Total alkaloidal content.
2. Total ash and acid-insoluble ash.
3. Moisture by the oven and toluene methods.
4. Total ether-soluble extract.
5. Volatile and non-volatile ether-soluble extracts.
6. Crude fiber.

1. The extraction of the total alkaloids was carried out by means of a Soxhlet extractor followed by shaking out with immiscible solvents to obtain the purified alkaloids as directed in the U. S. P. XI. They were determined volumetrically by treating with an excess of 0.02 *N* sulfuric acid and back titrating with 0.02 *N* sodium hydroxide solution. About 10 Gm. of the finely powdered drug was used as a sample each time. The assays were carried out on the leaves obtained from two crops, 1939 and 1940.

The alkaloidal content is indicated in Table I, which summarizes the results of the assays.

TABLE I.—ALKALOIDAL CONTENT

Sources of Leaves	Total Alkaloids, Per Cent by Weight 1939 Crop	1940 Crop
Wild plants	0.28	0.29
Cultivated plants, without fertilizer, from seeds of wild plants	0.32	0.36
Cultivated plants, with fertilizer, from seeds of wild plants	0.34	0.38
Cultivated plants, without fertilizer, from seeds obtained from the U. S. D. A.	0.14	0.19
Cultivated plants, with fertilizer, from seeds obtained from the U. S. D. A.	0.28	0.25

2. The total ash and the acid-insoluble ash were determined by the U. S. P. methods. The results are tabulated in Table II.

TABLE II.—TOTAL ASH AND ACID-INSOLUBLE ASH

Source of Leaves	Total Ash, Per Cent 1939 Crop	1940 Crop	Acid-Insoluble Ash, Per Cent 1939 Crop	1940 Crop
Wild plants	16.86	16.85	0.79	0.56
Cultivated plants, without fertilizer, from seeds of wild plants	10.11	9.41	0.65	0.43
Cultivated plants, with fertilizer, from seeds of wild plants	11.52	10.28	0.11	0.19
Cultivated plants, without fertilizer, from seeds obtained from the U. S. D. A.	14.63	10.24	1.09	0.27
Cultivated plants, with fertilizer, from seeds obtained from the U. S. D. A.	15.08	13.23	0.54	0.31

3. The moisture was determined on a 10-Gm. sample by the oven method of the U. S. P. and on a 20-Gm. sample by the toluene method. The results are given in Table III.

TABLE III.—MOISTURE CONTENT

Source of Leaves	Moisture Content, Per Cent Oven Method 1939 Crop	1940 Crop	Toluene Method 1939 Crop	1940 Crop
Wild plants	10.48	7.71	10.20	9.25
Cultivated plants, without fertilizer, from seeds of wild plants	13.86	10.71	12.40	10.10
Cultivated plants, with fertilizer, from seeds of wild plants	13.20	10.64	10.80	9.25
Cultivated plants, without fertilizer, from seeds obtained from the U. S. D. A.	13.47	10.69	11.20	10.50
Cultivated plants, with fertilizer, from seeds obtained from the U. S. D. A.	12.45	10.49	10.80	9.50

4. The total ether-soluble extract was determined by the U. S. P. method; the results are given in Table IV.

TABLE IV.—TOTAL ETHER-SOLUBLE EXTRACT

Source of Leaves (1939 Crop Only)	Yield, Per Cent
Wild plants	4.37
Cultivated plants, without fertilizer, from seeds of wild plants	4.76
Cultivated plants, with fertilizer, from seeds of wild plants	4.80
Cultivated plants, without fertilizer, from seeds obtained from the U. S. D. A.	4.82
Cultivated plants, with fertilizer, from seeds obtained from the U. S. D. A.	7.23

5. The volatile and non-volatile ether extracts were determined by heating the total ether-soluble extracts at 110° C.; the results are given in Table V.

TABLE V.—VOLATILE AND NON-VOLATILE ETHER-SOLUBLE EXTRACTS

Source of Leaves (1939 Crop Only)	Ether-Soluble Extracts, Per Cent Volatile	Non-Volatile
Wild plants	0.22	4.15
Cultivated plants, without fertilizer, from seeds of wild plants	1.32	3.53
Cultivated plants, with fertilizer, from seeds of wild plants	0.21	4.59
Cultivated plants without fertilizer, from seeds obtained from the U. S. D. A.	0.22	4.60
Cultivated plants, with fertilizer, from seeds obtained from the U. S. D. A.	1.32	5.99

6. The U. S. P. method for crude fiber was carried out with the results shown in Table VI.

TABLE VI.—CRUDE FIBER

Source of Leaves (1939 Crop Only)	Yield, Per Cent
Wild plants	7.85
Cultivated plants, without fertilizer, from seeds of wild plants	8.55
Cultivated plants, with fertilizer, from seeds of wild plants	8.90
Cultivated plants, without fertilizer, from seeds obtained from the U. S. D. A.	8.36
Cultivated plants, with fertilizer, from seeds obtained from the U. S. D. A.	8.64

SUMMARY

1. The alkaloidal content of wild Puerto Rican stramonium averages 0.29%, based on results for each of two successive years.

2. Cultivated plants grown from seeds of wild stramonium produced a higher percentage of total alkaloids than the wild plants even when no fertilizer was added.

3. Commercial fertilizer increased the yield of alkaloids but not as much as cultivation alone did.

4. Seeds from the continental United States produced a lower yield than native seeds. The application of fertilizer increased the yield but not up to that of the native plants.

5. The total ash content in the wild plants of both years' leaves was notably higher than in any of the cultivated plants.

6. Fertilized plants yielded more ash than the unfertilized plants, probably due to the salts of the fertilizer.

7. The wild plants contained a higher percentage of acid-insoluble ash than the cultivated plants in almost all cases.

8. The unfertilized plants produced a higher percentage of acid-insoluble ash than the fertilized plants in the majority of cases.

9. The moisture content of the wild plant leaves was much lower than that of any of the cultivated plants, which were uniform, presumably due to the irrigation that was necessary.

10. The wild plants produced the least amount of total ether-soluble extract and crude fiber.

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The Potency and Stability of a New Extract of *Convallaria majalis* Leaves*

By James R. Weeks,† Donald R. Mathieson and Harald G. O. Holck

During the past few years there has been a renewed interest in therapy using standardized extracts of *Convallaria majalis* leaves, at first in Europe (1, 2, 3), and then in the United States (4, 5). Our preparation[1] was made in May, 1940, by a method similar to that described by Straub (6). It consisted of an aqueous extract of the leaves, treated with ferric hydroxide to remove tannins and gums, and then concentrated under reduced pressure until 1 Gm. of the extract was equivalent to 30 Gm. of dried leaves.

The potency and stability of the extract, after storage under various conditions for five months, were determined by comparison with U. S. P. XI Reference Tincture of Digitalis (1 cc. = 1 U. S. P. Digitalis Unit) by the 18-hr. frog method. At the time of the first assay, which was seven months after the extract was manufactured, two samples

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[1] This preparation was furnished by Dr. C. W. Sondern of George A. Breon and Company, Kansas City, Mo.

of the extract and two of a 10% solution of the extract in distilled water were set aside, one of each being stored in a refrigerator and the other at room temperature, all protected from light.

EXPERIMENTAL

Male frogs (*Rana pipiens* Schreber), weighing from 16 to 30 Gm., were stored in running water about 1 cm. deep at 10° C. for one week prior to use. Twenty-four hours before injection, the frogs were transferred to the assay tank, held exactly at 20° C. Each frog was wiped gently with a towel, the urine expressed and the animal weighed to the nearest one-half gram. Injections were made into the pectoral lymph sac through the floor of the mouth. Observations were made at 18 (±½) hrs. after injection, a frog being considered dead when pain reflexes on pinching the toes were absent and subsequent examination showed that the heart was stopped.

The concentrations of the solutions injected were such that for all doses each frog received 0.02 cc. per Gm. of body weight. In addition, the alcohol content of the dilutions of the Reference Tincture of Digitalis was adjusted, by evaporation or addition of alcohol, to contain about 23% of alcohol by volume. Except for the first and last doses of convallaria extract in Table I, the doses are in equal logarithmic increments.

TABLE I.—FIRST ASSAY: COMPARISON OF CONVALLARIA EXTRACT AND U. S. P. XI REFERENCE TINCTURE OF DIGITALIS

Reference Tincture		Convallaria Extract	
Dose, Cc./Kg.	Mortality[a]	Dose, Mg./Kg.	Mortality[a]
1.78	0/15	17.8	0/15
2.24	4/15	22.4	1/15
2.82	6/15	25.1	5/15
3.55	13/15	28.2	8/15
4.47	15/15	31.6	12/15
5.62	15/15	35.5	14/15
...	...	44.7	15/15
LD_{50}:	2.80 ± 0.13 cc./Kg.		27.7 ± 0.7 mg./Kg.

0.1 Gm. of convallaria extract is equivalent to 10.1 ± 0.5 U. S. P. Digitalis Units.

[a] Mortality of controls, 4/66.

The first assay (Table I) was conducted in December, 1940, and the second (Table II) in May, 1941. The second assay was conducted in two sections on successive days. Using the logarithms of the doses, log LD_{50} (average lethal dose) was calculated by Kärber's method (7). The standard deviation of the logarithm of the LD_{50} was derived by the formula $\sqrt{\Sigma(pq/n)d^2}$, in which p = mortality as a fraction, $q = 1 - p$, n = number of animals and d = difference between logarithms of the successive doses (8). The standard error of the ratios of the LD_{50}'s of the standard to the extracts (standard error of the potency of the extracts) was calculated by the formula $\sqrt{S_1^2 + S_2^2}$, in which S_1 and S_2 are the

TABLE II.—SECOND ASSAY: COMPARISON OF U. S. P. XI REFERENCE TINCTURE OF DIGITALIS AND SAMPLES OF EXTRACT AND OF A 10% SOLUTION STORED FIVE MONTHS AT ROOM TEMPERATURE AND IN A REFRIGERATOR

PART 1

Reference Tincture		Convallaria Extract		
			Mortality[a]	
Dose, Cc./Kg.	Mortality[a]	Dose, Mg./Kg.	Refrigerated	At Room Temperature
2.37	0/12	23.7	0/12	0/12
2.82	0/12	28.2	0/12	0/12
3.35	3/12	33.5	2/12	2/12
3.98	5/12	39.8	5/12	7/12
4.73	5/12	47.3	8/12	8/12
5.62	10/12	56.2	11/12	12/12
6.68	12/12	66.8	12/12	12/12
LD_{50}:	4.41 ± 0.20 cc./Kg.		42.2 ± 1.8 mg./Kg.	40.4 ± 1.6 mg./Kg
Equivalent of 0.1 Gm. of convallaria extract in U. S. P. Digitalis Units:			10.4 ± 0.7	10.9 ± 0.7

PART 2

Reference Tincture		10% Solution of Convallaria Extract		
			Mortality[b]	
Dose, Cc./Kg.	Mortality[b]	Dose, Mg./Kg.	Refrigerated	At Room Temperature
...	...	23.7	1/12	1/12
2.82	0/12	28.2	2/12	0/12
3.35	3/12	33.5	0/12	1/12
3.98	4/12	39.8	4/12	4/12
4.73	9/12	47.3	7/12	11/12
5.62	9/12	56.2	9/12	10/12
6.68	11/12	66.8	12/12	12/12
LD_{50}:	4.34 ± 0.21 cc./Kg.		44.0 ± 2.1 mg./Kg.	41.6 ± 1.6 mg./Kg.
Equivalent of 0.1 Gm. of convallaria extract in U. S. P. Digitalis Units:			9.9 ± 0.7	10.4 ± 0.6

[a] Mortality of controls, 0/21.
[b] Mortality of controls, 1/25.

respective standard deviations of the LD_{50}'s expressed in per cent (8).

Our results show that the potency in all cases was well within the limits of the standard deviations; hence there has been no deterioration of the extract. A preliminary experiment, conducted in July, 1940, two months after the manufacture of the extract and five months before the first assay given above, showed that 0.1 Gm. of the extract was equivalent to approximately nine U. S. P. XI Digitalis Units. The absence of change, even when diluted 1 to 10 with water, is in line with the finding by Straub (6) that aqueous solutions of convallaria extract in sealed ampuls retained their full strength for four years.

SUMMARY

The potency of a concentrated, purified, aqueous extract of the leaves of *Convallaria majalis* was determined by comparison with digitalis on frogs. The extract and a 10% aqueous dilution thereof were found to be stable at room temperature during the course of five months.

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A Chemical Study of Oklahoma Plants

V. *Ephedra Nevadensis* Watson*

By Jess L. Beasley, Jr.,† and Loyd E. Harris‡

Fig. 1.—Habit photograph of *Ephedra Nevadensis*.[1]

Fig. 2.—*E. Nevadensis* showing staminate cones.[1]

Six species of Ephedra have been reported in North America, where they are found growing in the desert or semi-desert regions of Mexico, Colorado, Texas and Oklahoma (1). *Ephedra Nevadensis* was described by Watson (2) in 1879. Cross (3) reported in 1937 that collections of Ephedra were first made in Oklahoma, southwest of Hollis, in Harmon County, January 28, 1932.

Nagai (4) isolated the alkaloid ephedrine from *Ephedra vulgaris* in 1887. The use of this alkaloid in medicine has made it a drug of great importance during the past two decades. The chief sources of ephedrine are *Ephedra sinica* Stapf, *Ephedra equisetina* Bunge and other species of Ephedra (5)

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[1] By courtesy of Dr. G. L. Cross, Botany Department, University of Oklahoma.

in amounts varying from 1% to 2% of the air-dried plants. China supplies most of the drug material which is then extracted in the United States. Experiments on the cultivation of Asiatic Ephedra in the United States are being conducted. Hiner (6) has shown that there is a possibility of the growing of *Ephedra sinica*, on a commercial scale, in South Dakota.

Much attention has lately been given to the industrial synthesis of ephedrine and several patents have been granted, most of them originating in Germany (7).

Ephedra Nevadensis Wats. is found growing, in very limited numbers, in southwestern Oklahoma. It has been used as a treatment in kidney and venereal diseases by Indians and Mexicans. Some studies on the constituents of the plant found growing in California have been made. Terry (8) reported that no ephedrine or any alkaloid could be detected. Nielsen (9) and others reported the absence of ephedrine. Read and Feng (10) found nothing more than a possible trace of ephedrine; extracts did not give any rise in blood pressure.

No reports have been given on chemical studies of the Oklahoma plant. Since it is known that different cultural conditions, in some instances, will materially affect the nature of the chemical constituents of plants, it was believed worth while to make this study.

EXPERIMENTAL

The material used in this investigation was collected southwest of Hollis, Harmon County, Oklahoma, in early November, 1940. There had been no frosts or freezing weather up to that date. The green stems were the only part of the plant used for this examination. The material was allowed to dry in sacks, away from the sun and then reduced to a No. 20 powder before extraction.

Moisture and ash determinations were made on the powdered material, using the methods given in the United States Pharmacopœia, eleventh revision. The results are given in the following tables.

MOISTURE DETERMINATIONS

Weight of Sample, Gm.	Moisture, Per Cent
20	8.2
30	8.0
	8.1 Av.

ASH DETERMINATIONS

Weight of Sample, Gm.	Acid-Insoluble Ash, Per Cent	Total Ash, Per Cent
2.103	0.45	8.05
2.105	0.52	8.10

Extraction with Selective Solvents.—Samples of the ground material were extracted successively in a Soxhlet extractor with petroleum ether (b. p. 25–60° C.), anhydrous ether, chloroform, dehydrated alcohol and water. The residues remaining upon evaporation of the petroleum ether and ether were sticky and dark brown. The chloroform and alcohol extractions were gummy and dark green. The percentage of each extractive is listed in the following tabulation.

EXTRACTIVE CONSTITUENTS OBTAINED BY SELECTIVE SOLVENTS, PER CENT

Weight of Sample, Gm.	Petroleum Ether	Ether	Chloroform	Alcohol	Water
12.132	0.94	4.86	1.07	6.38	14.6
11.742	0.94	5.04	0.94	6.43	15.5

The residue from the alcohol extraction was treated with 50 cc. of water. (A 10-cc. portion of the aqueous solution was evaporated and calculations from this aliquot portion showed that two-thirds of the alcoholic residue had dissolved in water.) Drops of the amber-colored solution were tested on slides with the use of a microscope, using the following alkaloidal reagents: Wagner's reagent, potassium dichromate, picric acid, lead acetate, mercuric chloride and ammonium molybdate.

Positive results were obtained with ammonium molybdate and indications of precipitates were obtained with the first three of the reagents.

Tests for Ephedrine and Other Alkaloids.—A 20-Gm. sample was treated by the method outlined by Christensen and Hiner (6). The 250-cc. percolate was evaporated to 50 cc. Light, oily globules formed on top of the liquid and, when placed in a separatory funnel and left over night, a brown heavy oil-like material separated from the liquid. This oily liquid was drawn off, dried *in vacuo*, but did not result in a definite crystalline structure as shown under the microscope.

The ether-chloroform extraction, after separation of the oily material, was diluted to 100 cc. and divided into four parts. (*a*) One part was treated by the method of Chen (11), but a heavy clear oily liquid was obtained, which gradually darkened on exposure, where crystalline ephedrine hydrochloride should have appeared, had the alkaloid been present. (*b*) Another portion of it was treated in a similar manner, substituting sulfuric acid for hydrochloric acid, with similar results. (*c*) A third portion of the solution was tested by the method of Kelly (12) for the detection of small quantities of Ephedra alkaloids, with negative results. Color reactions for ephedrine as suggested by Pesez (13) also gave negative results.

SUMMARY

The green stems from the plant, *Ephedra Nevadensis* Wats., growing in southwestern Oklahoma, have been examined. In the preliminary analysis the percentages of moisture, total ash and acid-insoluble ash were determined. Samples of the powdered material were extracted by selective solvents. Tests for the presence of ephedrine were negative.

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Effect of Picrotoxin on the Blood Potassium of Anesthetized Animals*

By Carlos E. Cardini and Maria E. Serantes

Picrotoxin strongly antagonizes the effects of narcotics; it increases respiratory tonus, restores consciousness, etc. Advantage has been taken of this action in the treatment of barbiturate poisoning. Maloney and collaborators (1), Rosenthal and Wallach (2), and Marshall and co-workers (3), have, among others, studied this question.

During anesthesia, changes appear in the titers of the inorganic constituents of the blood, the most prominent of these being a marked decrease in plasma potassium (Marenzi and Gerschman (4), 1933). It was considered of interest to study the narcosis-induced decrease in plasma potassium as affected by the suppression of narcosis with picrotoxin. It is also to be remembered that picrotoxin, along with its antinarcotic action, possesses convulsant properties capable by themselves of inducing variations in the plasma potassium of normal dogs.

EXPERIMENTAL

The anesthetic selected, chloralose, was administered intravenously to dogs at the rate of 10 ml. of an 8% solution per Kg. After deep anesthesia had been induced, a 0.5% solution of picrotoxin (Merck) in distilled water was injected intravenously, rapidly or gradually, in a dosage varying from 0.10 mg. to 1 mg./Kg. for rapid injections, from 0.25 to 0.35 mg./Kg. in prolonged administration, distributed over 10 to 20 min. Blood was obtained from the carotid artery at intervals noted in the curves. Potassium was determined by the method of Marenzi and Gerschman (4), 1932, using blood deproteinized with trichloroacetic acid. Potassium was determined directly in plasma and whole blood. The red cell potassium content was calculated from cell volume determinations and from plasma and whole blood analyses. Red cell concentration or contents is of very little significance even when water determinations and R. B. C. counts are available.

RESULTS

Rapid Administration.—Doses of 0.10 mg. and 0.12 mg./Kg. do not induce the disappearance of narcosis. Antagonistic action begins to be evident with doses above 0.15 mg. There is a latent period which for this dosage varies between 8 and 10 min. As a rule, in 20 to 30 min., convulsions begin to appear which last to the end of the experiment. The animal is conscious throughout the entire experiment, which lasts more than an hour. With a dose of 0.25 mg./Kg., consciousness is regained within 8 to 20 min., but rather severe convulsions appear 10 min. after the injection. With a dose of 0.50

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mg., the picture is similar. With 1.0 mg., there is no latent period; immediate violent convulsions set in and the animal becomes conscious. The convulsions persist to the end.

Gradual Administration.—The results are similar to those obtained with massive dosage. In one series of experiments 0.015 mg./Kg. was injected once a minute for 20 min., a total dosage of 0.30 mg./Kg. The animal awoke in 15 to 20 min., and slight convulsions were observed. In another series, 0.025 mg./Kg. was injected every minute for 10 min.; total dosage 0.25 mg. The animal awoke at the end of the last injection and convulsions were observed that lasted until the end of the experiment.

Variations in Plasma Potassium.—The most significant experiments are represented by curves (Figs. 1, 2, 3). In Fig. 1 the descending curve resulting from the action of the anesthetic is to be

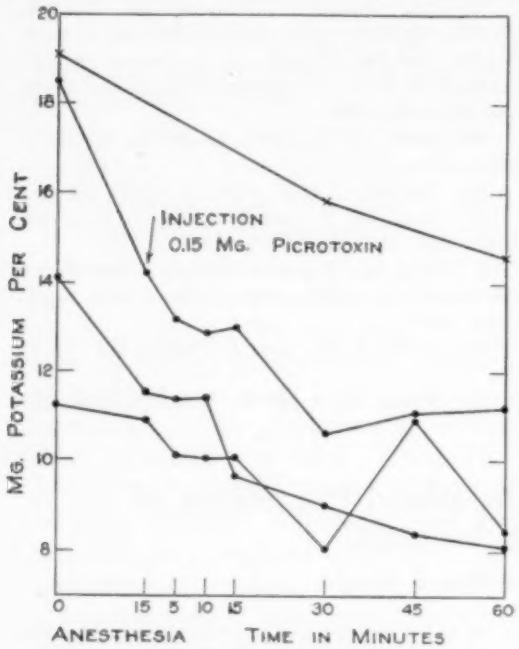

Fig. 1. (X-curve without picrotoxin).

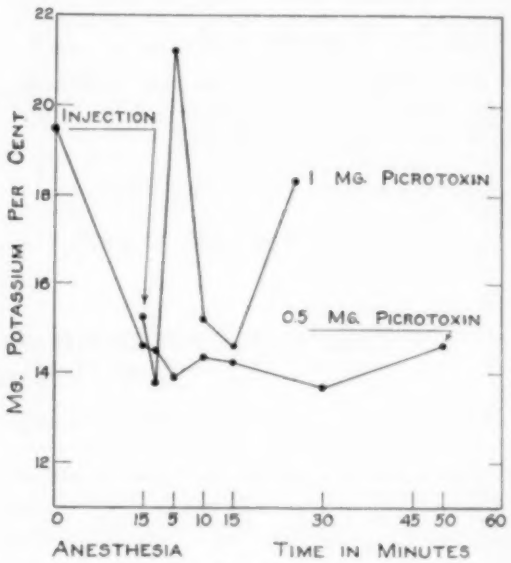

Figure 3.

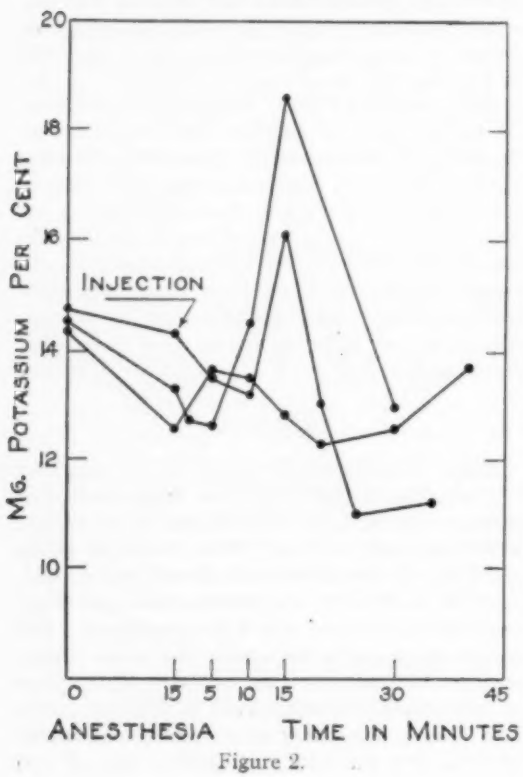

Figure 2.

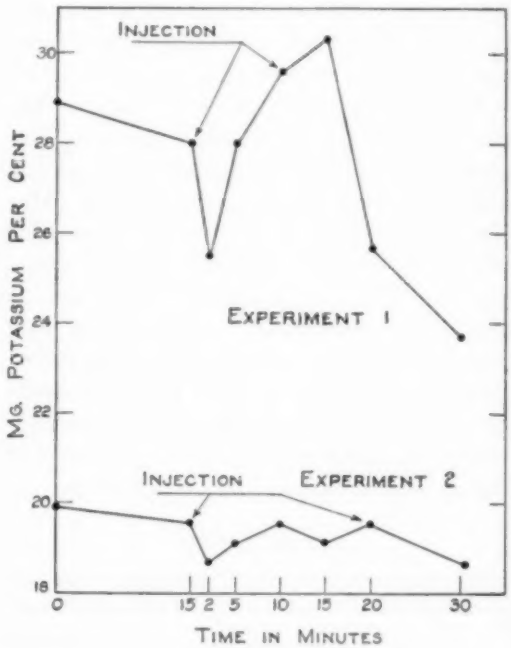

Figure 4.

TABLE I.—THE PICROTOXIN–CHLORALOSE ANTAGONISM

				Variation in Potassium in Whole Blood							Variations in Potassium Referred to Original Cell and Plasma Volume		
Exp. No.	Wt. of Dog, Kg.	Amt. of Picrotoxin Injected, Mg./Kg.	Time, Min.	Cell Volume	Plasma Volume	Blood Potassium, Mg. %	Plasma Potassium, Mg. %	Blood Cell Potassium,[a] Mg. %	Cell Volume Potassium, Mg.	Plasma Volume Potassium, Mg.	Potassium in Cells, Mg. %	Potassium in Cell Volume, Mg.	Potassium in Plasma Volume, Mg.
1	24	1.00	0	57.57	42.43	18.8	15.3	21.4	12.3	6.5	21.4	12.3	6.5
			2	51.92	49.08	26.6	13.7	40.2	20.9	5.7	36.2	20.8	5.8
			5	56.70	43.50	22.6	21.2	23.7	13.4	9.1	23.7	13.7	9.0
			10	59.00	41.00	20.2	15.2	23.7	14.0	6.2	24.0	13.8	6.5
			15	59.18	40.82	19.5	14.5	23.1	13.6	5.9	23.3	13.4	6.2
			25	58.25	41.75	21.1	18.3	23.1	13.4	7.5	23.8	13.4	7.7
2	23.5	1.00	0	39.96	60.04	20.3	18.0	23.4	9.3	10.8	23.4	9.3	10.8
			2	50.51	49.49	27.2	25.9	28.6	14.4	12.7	29.3	11.7	15.4
			5	53.57	46.43	33.0	27.8	37.4	20.1	12.8	46.1	18.4	14.7
			10	53.39	46.61	30.1	30.1	30.1	16.0	14.0	30.8	12.0	18.0
			15	54.20	45.80	28.8	26.8	30.5	16.5	12.2	32.0	12.7	16.1
			25	57.00	43.00	30.5	29.2	31.6	18.0	12.2	32.6	13.0	17.4
3	6.0	0.25	0	44.97	55.03	23.9	17.1	34.4	15.4	9.4	34.4	15.4	9.4
			7	41.49	58.51	21.2	13.9	31.6	13.0	8.1	30.2	13.6	7.6
			12	48.01	51.99	20.3	15.4	25.8	12.3	7.9	26.1	11.9	8.5
			20	48.35	51.65	21.9	15.7	28.5	13.7	8.1	29.6	13.3	8.6
			25	48.58	51.42	21.2	14.4	29.3	14.2	6.9	29.6	13.3	8.0
			30	47.97	52.03	22.4	16.0	29.4	14.1	8.3	30.3	13.6	8.8

[a] Calculated from plasma and whole blood, potassium analyses and all volume determinations.

noted; the injection of 0.15 mg. of picrotoxin does not modify this drop. This dosage is of major interest because it shows that the antagonistic action of the drug does not modify the drop in potassium; it is the dose which restores consciousness without producing convulsions.

With greater doses the restoration of consciousness is accompanied by violent convulsions; these convulsions coincide with an abrupt increase in potassium. This is not invariably so, for in some instances (Fig. 3) with a dose of 0.5 mg., which induced violent convulsions, the fall in potassium continued. The rarity of these findings leads one to attribute them to a factor other than the direct effect of picrotoxin.

In prolonged dosage (Fig. 4) there is a small drop at the beginning of the injection, then an ascending period, but in a manner much less pronounced than following the massive injection of an equal quantity of picrotoxin. The last decline is less marked than that induced by the anesthetic alone.

Variations in Distribution of Potassium between Blood Cells and Plasma.—Doses of 0.25 mg. and 1 mg. of picrotoxin per Kg. were selected for a study of the possible interchange of potassium between the blood cells and plasma. Both dosages induced definite changes in plasma potassium. The results obtained did not appear to depend on the size of the dose (Table I).

It was observed in these experiments that the blood cell volume underwent significant but variable changes. In experiments 1 and 3, there was a decrease in cell volume, followed by an increase and finally a return to its original value. In experiment 2, the cell volume increased throughout the experiment.

Significant changes in the erythrocyte count, erythrocyte water content and cell volume are known to occur in the intact animal as a result of various procedures such as exercise and epinephrine injection. The data available in these experiments are insufficient to determine these changes in the erythrocytes of the circulating blood and hence an accurate expression of erythrocyte potassium exchanges is impossible.

Large increases in plasma potassium concentration were accompanied by a significant increase in red cell potassium either on a concentration (mg./100 cc. of cells) or a cell volume content (mg./100 cc. of whole blood) basis. This increase in red cell potassium on both a concentration and a cell volume content basis apparently results from a transfer of potassium from the plasma into the cells and the addition of a large number of erythrocytes to the circulating blood due to the contraction of certain organs.

CONCLUSIONS

A clean-cut antagonism was shown between picrotoxin and the narcotic action of chloralose.

In each instance the animal awoke and remained awake throughout the course of the experiment. Along with this action picrotoxin retained its marked convulsant activity.

The smallest dose which induced suppression of narcosis without causing convulsions was 0.15 mg./Kg.

Many authors have correlated the convulsant action of picrotoxin with its antagonistic action against narcotics (Krautz and collaborators (5)); the present results show only that these actions do not necessarily occur simultaneously.

The decrease in plasma potassium, which normally occurs during anesthesia, is modified only when convulsions are induced, which would indicate that the suppression of narcosis does not modify this decrease but renders it less intense.

At the beginning of convulsions, marked changes in the blood appear.

In most experiments there is an abrupt increase in potassium, coinciding with the beginning of muscular contractions; the potassium values then become lower as this behavior continues.

This abrupt increase is very similar to that produced by the intravenous administration of epinephrine. It may in part be attributed to the liberation of muscle potassium during the spasms and to the indirect effect of the discharge of epinephrine by bulbar action, which also produces a hyperglycemia, already emphasized by various workers studying the action of picrotoxin. This would be in agreement with the mechanism of the regulation of potassium as demonstrated by Houssay, Marenzi and Gerschman (6), 1936. It is interesting to note that picrotoxin displays this antagonism in a latent period of 5 or more min., except in very high doses where the effect is almost immediate.

The changes in the whole blood potassium are principally referable to changes in the potassium concentration in the cells and cell volume, because the change in plasma potassium content in terms of whole blood volume is very small. Possibly during the muscle and organ contractions, there is an abrupt entry of red cells into the blood; in addition, a transfer of potassium to the cells very probably occurs, because a constant increase greater than the experimental error inherent in this indirect determination was observed in the red cell potassium concentration.

Results obtained with other inorganic constituents prove that the action of picrotoxin does not greatly modify the variations in them induced by anesthesia, as studied by Marenzi and Gerschman (7) (1933), except that magnesium here tends to increase, whereas ordinarily it decreases during anesthesia.

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The Relation of Alkaloidal Chemistry to Inorganic, and the Use of Bromauric Acid as a Reagent for Inorganic Microcrystal Tests*

By Charles C. Fulton†

RELATIONSHIPS

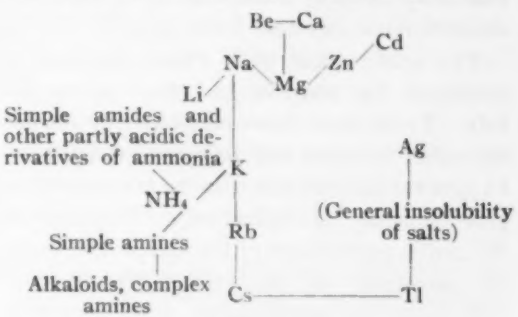
The so-called "alkaloidal reagents" are really amine precipitants, and precipitate other complex amines as readily as the alkaloids. Simple amines are precipitated by the more general of these reagents. Ammonium, even, is precipitated by some of them.

On the inorganic side, ammonium is very closely related to potassium, the latter showing almost the same solubilities of salts. Potassium in turn is related to the heavier alkali metals, rubidium and cæsium, and to the lighter, sodium and lithium. Rubidium is precipitated by about a third or more of the "alkaloidal reagents"—some 200 formulas, utilizing at least 75 different compounds as precipitating agents—and cæsium is precipitated by most of them. Thallium (univalent) is related to cæsium and is also precipitated by these reagents, but here we get into a general insolubility of most salts, as with silver.

Although sodium is closely related to potassium in most respects, there is quite a gap between them in the properties we are now considering. In fact, practically speaking, sodium is not precipitated by any of the "alkaloidal reagents" from aqueous solutions. However, the writer (1) has previously shown that the amine compounds reach an extreme insolubility in syrupy phosphoric acid; and this is true also of the inorganic precipitates. In a test drop of this acid, not only sodium, but also lithium, magnesium, zinc and cadmium are precipitated in a few compounds, and in particular as bromaurates. (Paradoxically, lithium is even more readily precipitated as bromaurate than sodium, and magnesium more readily than zinc or cadmium.) With a high concentration of the precipitating compound, bromaurate crystals can also sometimes be obtained with calcium and beryllium.

Not all the elements or metals have been investigated, by any means, but apparently the further we get away from the heavy alkali metals the more difficult it is to get bromaurates or any of the other insoluble compounds characteristic of "alkaloidal" precipitation.

The relationships mentioned above are indicated in the accompanying diagram:



BROMAURIC ACID

Bromauric acid, or "gold bromide," $HAuBr_4$, is very sensitive to most alkaloids, but is not as general a precipitant in aqueous solution as the complex oxygen acids and some other reagents. It does not precipitate ammonium from water. In syrupy phosphoric acid, however, it becomes virtually a reagent for basic nitrogen, even in compounds predominately acidic. The acid acts not only to decrease the solubility of the compounds formed, but also to suppress any acidic characteristics in the substances tested. The greatest value of such a reagent is, naturally, not with alkaloids which can be precipitated by a host of other reagents, but with the simpler amines, and amine derivatives that are partly acidic; *e. g.*, amides, amino acids and other organic acids which contain nitrogen with some residual basic properties.

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Likewise in inorganic chemistry the value of $HAuBr_4$ in H_3PO_4 is greatest in the microcrystal tests it gives for lithium, sodium, magnesium and zinc. Common as sodium and magnesium are, good tests for them are not plentiful, so the value of this reagent is all the greater.

For the more easily precipitated alkali metals, and ammonium, diluted phosphoric or sulfuric acid can be used as solvents for $HAuBr_4$, giving reagents with different properties.

The tests of this article are made by adding a drop of the reagent directly to a little of some powdered salt of the metal or base to be identified. A cover glass is applied and examination made under the microscope. The bromaurates are easily distinguished by their colors from any particles of the original salt remaining undissolved, or other crystals that may possibly form. In most cases the characteristic crystals form quickly.

The acid radical with which the base is combined has considerable effect on the result. Trials have been made chiefly on the chlorides, sulfates and primary phosphates. In general the sulfates may be considered to give "normal" precipitation. Phosphate as the acidic constituent of the salt will increase the sensitivity of the test, while chloride will make it considerably less sensitive. Acetate also decreases the sensitivity of the test. Bromides are similar to chlorides. Salts such as iodides, which react at once with the reagent, cannot be used. Nitrates soon react with the reagent made with syrupy H_3PO_4, but can be used at least with the weaker reagents. Bisulfates and secondary and tertiary phosphates can be used. The lesser sensitivity of chlorides and bromides as compared to sulfates and phosphates is not a disadvantage in all cases, for if precipitation is still obtained the crystals are usually larger.

For organic identifications over the whole field of compounds of basic nitrogen, a series of "gold bromide" reagents is very desirable, in fact essential. $HAuBr_4$ in H_3PO_4 has the greatest precipitating power, but is chiefly useful with compounds rather difficult to precipitate. Bromaurates of easily precipitated substances, such as the alkaloids, are usually too insoluble in H_3PO_4 for good results, do not crystallize readily enough or crystals are too minute. $HAuBr_4$ in concentrated HCl will probably provide more good microcrystal tests than any other reagent known. The various "gold bromide" reagents, utilizing H_3PO_4, diluted H_2SO_4, HBr, HCl, water and acetic acid, differ not merely in the solubilities of the bromaurates in the test solution, and in the readiness with which crystallization occurs, but also in the actual forms and kinds of crystals produced. The crystals form so readily, with so many different substances, and are so highly characteristic, that a series of 8 or 10 different $HAuBr_4$ reagents used both on dry substances and on aqueous solutions will probably provide more good microcrystal tests for all kinds of compounds of basic nitrogen than any equal number of other reagents, utilizing other precipitating compounds.

A series of different "gold bromide" reagents can be used in inorganic chemistry also, as described below. However, $HAuBr_4$ in syrupy H_3PO_4 is the outstanding reagent. The reagents can be applied to aqueous solutions for inorganic chemistry also, and the heavier alkali metals and ammonium can be so precipitated, but not the lighter metals. The latter require strong phosphoric acid for the test drop. In some cases the compound might first be dissolved in syrupy phosphoric acid and the reagent applied; but a fairly high concentration of both base and $HAuBr_4$ is required, and is best obtained in localized spots by applying the reagent to a little of the dry salt.

The tests have a limited applicability to mixtures. A reactive base can be identified in the presence of material with which the reagent does not react. If two reactive bases are present a reagent can often be chosen which will affect only the one more easily precipitated.

Most of the tests are fairly sensitive, but they are intended for identification, not for the detection of minute amounts. They belong to that class of tests covered by Chamot and Mason in their "Handbook of Chemical Microscopy" (2), except that the large majority of current tests depend on the mixing of two aqueous solutions.

BROMAURIC ACID IN SYRUPY PHOSPHORIC ACID ($HAuBr_4$ IN H_3PO_4)

Gold chloride crystals ($HAuCl_4 \cdot 3H_2O$)	1	Gm.
HBr (40%)	1.5	cc.
Water	1.0	cc.
Syrupy (85%) H_3PO_4		
To make	20	cc.

The formula for this reagent was originally given (1) without the 1 cc. of water. It is then saturated with $HAuBr_4$, with some undissolved crystals, as this compound itself is not highly soluble in syrupy H_3PO_4. It has been found much better to use just enough water to hold all the $HAuBr_4$ in solution. The reagent can be made with $AuBr_3$ + HBr, or with $HAuBr_4$ crystals, both of which are commercially obtainable; but since "gold chloride" (chlorauric acid) is the most commonly used gold salt, and seems to work just as well, it is used to make the reagent.

Uses.—Identifying magnesium, zinc, cadmium, sodium, lithium, potassium, ammonium, rubidium, cæsium and innumerable organic compounds of nitrogen of slight basic properties.

CRYSTALS

Magnesium.—Beautiful crystals, orange to deep red hexagonal plates, are obtained from the chloride, either $MgCl_2 \cdot 6H_2O$ or dried $MgCl_2$, and from the phosphate, $MgH_4(PO_4)_2$ (Fig. 1). Good crystals are also obtained from the nitrate, $Mg(NO_3)_2 \cdot 6H_2O$, before decomposition sets in. The sulfate, $MgSO_4 \cdot 7H_2O$, yields smaller and more numerous crystals.

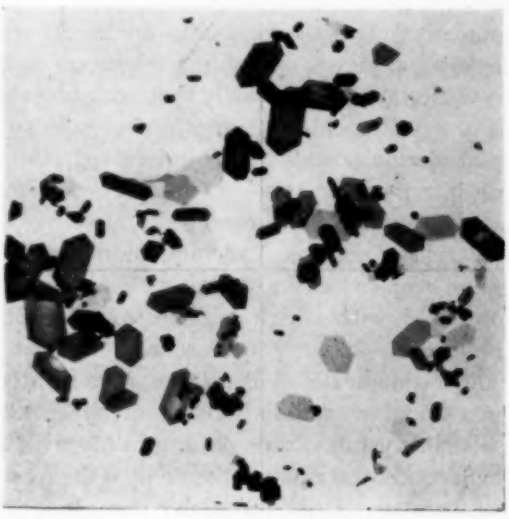

Fig. 1.—Magnesium bromaurate (magnesium gold bromide). Obtained with $HAuBr_4$ in syrupy H_3PO_4, and $MgCl_2.6H_2O$. Photomicrograph by John B. Dalton, Police Department, St. Paul, Minn.

Zinc.—The sulfate gives crystals related to those of magnesium, but lighter, more yellow-orange in color and mostly diamond-shaped, rather than hexagonal, when first formed. The crystals from the phosphate are very small, minute orange grains, or diamond-shaped or hexagonal platelets. They are better if the H_3PO_4 is a little diluted with water. The chloride and acetate cannot be depended on to yield any crystals.

Cadmium.—The sulfate and phosphate yield small brown-red plates, on or very close to the undissolved particles.

Sodium.—The sulfate, Na_2SO_4, and the phosphate, $NaH_2PO_4 \cdot H_2O$, yield crystals readily, orange splinters or thin rods at first, tending to become thin brownish yellow prisms (Fig. 2). These crystals have a fairly well-marked dichroism with polarized light. They are just obtained from the bromide or acetate, and cannot be obtained at all from the chloride with this strength of reagent, but one more concentrated in $HAuBr_4$ will give them.

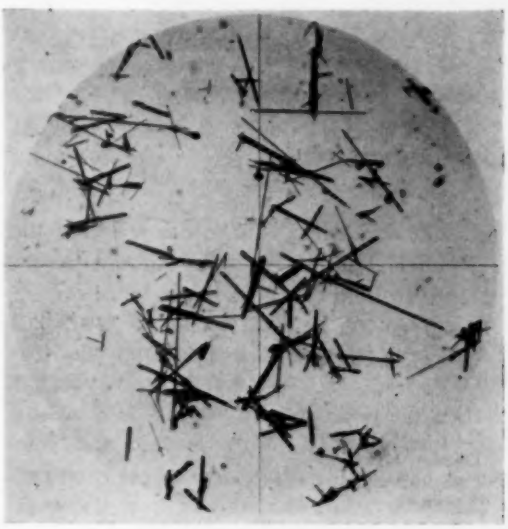

Fig. 2.—Sodium bromaurate (sodium gold bromide). Obtained with $HAuBr_4$ in syrupy H_3PO_4, and Na_2SO_4. Photomicrograph by John B. Dalton, Police Department, St. Paul, Minn.

Lithium.—The crystals are similar to those of sodium, but are best obtained from the chloride. The sulfate and phosphate do

not dissolve readily in the reagent, but the crystals soon form around the undissolved material.

Potassium.—There are several different forms of crystals, but the precipitate is characterized by small, deep red, rectangular grains. The crystals are somewhat larger with the chloride than with the sulfate or phosphate, but any convenient salt can be used.

Ammonium.—There are several different forms of crystals, the precipitate being characterized by orange spearhead and diamond-shaped plates. The chloride gives larger crystals than the sulfate or phosphate. Any convenient salt can be used.

Rubidium.—The crystals resemble those with ammonium.

Cæsium.—There is an opaque red precipitate, amorphous or too minutely crystalline for the shape of the crystals to be distinguished. Gradually fairly large, elongate, yellow-orange plates grow out from the amorphous masses. Also small to minute diamond-shaped plates, orange-yellow, form around the edges of the amorphous precipitate.

A SERIES OF "GOLD BROMIDE" REAGENTS

1. Concentrated $HAuBr_4$ in H_3PO_4.

$AuBr_3$	1 Gm.
HBr (40%)	0.4 cc.
Water	0.6 cc.
Syrupy H_3PO_4	
To make	7.5 cc.

See that the $AuBr_3$ is in solution before adding the H_3PO_4. The water should be just enough to hold the $HAuBr_4$ in solution.

2. $HAuBr_4$ in H_3PO_4 (see p. 179).

3. $HAuBr_4$ in (3 + 1) H_3PO_4.

$HAuCl_4$ crystals	1 Gm.
HBr (40%)	1.5 cc.
Diluted H_3PO_4 (3 vols. syrupy H_3PO_4 to 1 vol. water)	
To make	20 cc.

4. $HAuBr_4$ in (1 + 1) H_3PO_4. "Potassium reagent." Like the preceding, but using more dilute H_3PO_4 (equal vols. syrupy H_3PO_4 and water).

5. $HAuBr_4$ in (2 + 3) H_2SO_4. "Ammonium reagent."

$HAuCl_4$ crystals	1 Gm.
HBr (40%)	1.5 cc.
Diluted H_2SO_4 (2 vols. conc. H_2SO_4 to 3 vols. water)	
To make	25 cc.

6. $HAuBr_4$ in HBr. "Amine reagent."

$HAuCl_4$ crystals	1 Gm.
Water	2.65 cc.
HBr (40%)	24 cc.

If kept well stoppered these reagents are, so far as known, entirely permanent. The series might be extended even further for organic chemistry.

The first of the foregoing reagents gives the limit of the range of precipitation by $HAuBr_4$. Thus in determining if there is any very slight tendency toward the formation of a bromaurate, it must sometimes be used instead of the usual $HAuBr_4$ in H_3PO_4, No. 2. Its disadvantages, however, are greater cost and the much deeper color of the solution. The colors of the crystals, which are often very characteristic, are not easily seen when the reagent solution is so deeply colored. This reagent, No. 1, gives the sodium crystals with the chloride, and more readily than No. 2 with the bromide, acetate and nitrate. With the nitrate, good sodium bromaurate crystals are formed before decomposition begins; afterward bright red blades are formed in the solution around the outside of the area of decomposition. The zinc crystals with the sulfate are bright red hexagonal plates, resembling the magnesium crystals much more closely than with reagent No. 2. Zinc chloride gives orange splinter-plates, zinc acetate yields orange-red plates of intermediate type. These crystals are fairly soluble in the reagent as affected by chloride or acetate. Calcium chloride yields some deep red sticks or grains. They can be observed, with a high transmission of light, imbedded in the undissolved material. The bromaurate of beryllium is evidently just about as soluble as that of hydrogen, so that crystals can be obtained only with a high concentration, and then only if the room temperature is not too high. Long orange splinters were obtained with the sulfate. The phosphate of beryllium was not at hand for trial.

The crystals with reagent No. 2 have already been described.

No. 3 no longer gives crystals with magnesium chloride, zinc sulfate or cadmium sulfate or phosphate. The crystals with magnesium sulfate and zinc phosphate are, however, larger and better than with the preceding reagent. Potassium gives slender orange rods at first, gradually also deep red grains and prisms. Rubidium gives good crystals, orange diamond-shaped plates and dark orange sticks, many saw-edged or spear-headed with orange plates.

No. 4, "potassium reagent," is intended to precipitate potassium but not sodium. The distinction is not quite absolute, as in order to leave the reagent sensitive enough to precipitate potassium as chloride, we have to allow for a slight effect on sodium as phosphate. If considerable sodium phosphate ($NaH_2PO_4 \cdot H_2O$) is used, some sodium crystals may be found among the undissolved particles. They tend to dissolve in the solution. Na_2SO_4 gives no crystals. The potassium crystals are slender orange rods or rod-plates, and are obtained with the bromide, acetate and nitrate, as well as the chloride, sulfate and phosphate. This reagent no longer yields crystals with magnesium sulfate or lithium chloride or sulfate, or zinc sulfate. It does, however, yield the bromaurates of magnesium and lithium from their phosphates.

No. 5, "ammonium reagent," is intended to precipitate ammonium but not potassium. The chloride and sulfate of potassium give nothing. The phosphate, KH_2PO_4, gives nothing immediately, even when used in large amount, but on standing for some time orange-red prisms form, mostly small and closely associated with the undissolved particles. Ammonium gives a dimorphous precipitate. With the phosphate and sulfate there are small orange grain-plates, and also larger dark, irregular stick-crystals. With the chloride the crystals are larger, the grain-plates redder and tending to the diamond shape. The precipitate with NH_4Cl is shown in Fig. 3. This reagent is a little more sensitive to magnesium and zinc than the preceding one, and gives crystals with their phosphates. Sodium and lithium are barely precipitated from their phosphates.

Fig. 3.—Ammonium bromaurate (ammonium gold bromide). Obtained with $HAuBr_4$ in (2 + 3) H_2SO_4, and NH_4Cl. Photomicrograph by John B. Dalton, Police Department, St. Paul, Minn.

No. 6, "amine reagent," precipitates methylamine and ethylamine, and all more complex, fully basic amines, but not ammonium, as the chloride. Ammonium sulfate or phosphate will yield a few crystals, orange needles, splinters or rods, near the undissolved material. These tend to dissolve in the solution. Potassium phosphate likewise yields a few orange-red rods or splinters among the undissolved particles. Sodium, lithium, magnesium and zinc yield no crystals. Rubidium gives a characteristic precipitate of small crystals, orange to red: grains, rods, prisms and diamond-shaped plates. Cæsium gives a black precipitate of minute grains or little sticks, sometimes in star formation. The crystals with methylamine and ethylamine are shown in Figs. 4 and 5. Those with methylamine are brown plates, usually square. Crystals in the same field differ markedly in depth of color, although they show no dichroism with polarized light. Those with ethylamine are of two kinds. First are large orange-red, square-cut plates. On standing there develop also large thick grain-plates, mostly hexagonal or coffin-shaped, deep red to nearly black.

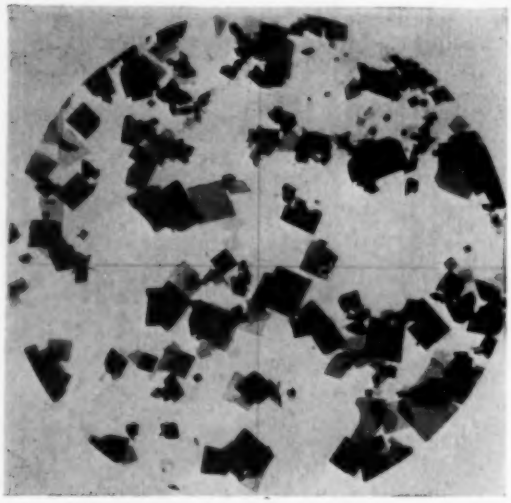

Fig. 4.—Methylamine bromaurate (methylamine gold bromide). Obtained with $HAuBr_4$ in HBr, and methylamine hydrochloride. Photomicrograph by John B. Dalton, Police Department, St. Paul, Minn.

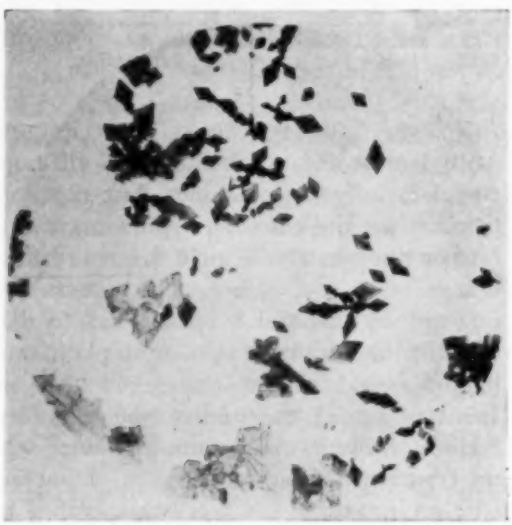

Fig. 5.—Ethylamine bromaurate (ethylamine gold bromide). Obtained with $HAuBr_4$ in HBr, and ethylamine hydrochloride. Photomicrograph by John B. Dalton, Police Department, St. Paul, Minn. (*Note:* This photograph was taken with only half the magnification used for the others, in order to get both kinds of ethylamine crystals in the field.)

CONCLUSIONS

1. The chemistry of the alkaloids, as regards precipitation and microcrystal tests, is closely related to that of rubidium and cæsium. Some of the "alkaloidal reagents" also precipitate ammonium and potassium. A few newer reagents, which extend the field covered in organic chemistry to even the simplest and most feebly basic amine derivatives, will precipitate even sodium, lithium, magnesium, zinc and cadmium, at least from syrupy phosphoric acid.

2. Bromauric acid in syrupy phosphoric acid, the most valuable reagent for simple, feebly basic amine derivatives, is also useful in inorganic chemistry for identifying magnesium, zinc, cadmium, sodium, lithium, potassium, ammonium, rubidium and cæsium by microcrystal tests. It is applied directly to a little dry salt of the metal, or of ammonium, and is particularly valuable for magnesium and sodium (Figs. 1 and 2).

3. A series of "gold bromide" reagents is of great value for organic chemistry, and may be used for inorganic. $HAuBr_4$ in (1 + 1) H_3PO_4 precipitates potassium, but not sodium, except for a few crystals when considerable phosphate is used for the test. Similarly $HAuBr_4$ in (2 + 3) H_2SO_4, of the formula given, is a reagent for ammonium but not potassium (Fig. 3). $HAuBr_4$ in HBr, of the formula given, precipitates rubidium and cæsium, and methylamine, ethylamine and all more complex, fully basic amines, but not ammonium from its chloride (Figs. 4 and 5).

4. These tests are intended for identification, not for detection of minute amounts. Broadly speaking, they belong to the class of tests covered by Chamot and Mason in their "Handbook of Chemical Microscopy," Vol. II.

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Storage Factors Affecting the Deterioration Rates of Certain Chlorine Preparations of the Sodium Hypochlorite Type*

I. The Effect of Diffused Daylight

By Elmer M. Plein and L. Wait Rising

In the course of an investigation conducted on the problem of stabilizers for a chlorine preparation made by one of our western manufacturers, the writers learned that the deterioration rates for such preparations were subject to unexpected vagaries. The exploratory work indicated that not only are extremely rapid deterioration rates an actuality under certain conditions of use, but that the same preparations under other circumstances can be stable to an unsuspected degree. A study of the literature demonstrates, too, how little has been published concerning the effect of factors such as age on the chemicals which are the active agents of such preparations before they are put into solution for use, while in solution as reserve stock, and under other conditions of storage. The investigation clearly showed the possibility that many of the sodium hypochlorite and *p*-toluenesulfonchloramide types of antiseptics now in general use having a variety of purposes might be failing to a hazardous degree in their function because of unrecognized deterioration. Hospitals, manufacturers, the Drug Deterioration Committee of the American Association of Colleges of Pharmacy, and users of the substances other than those directly connected with the public health professions, such as restaurants, taverns, public baths and laundries, are all seriously concerned with the instabilities of such preparations and the things that might be done to alleviate instability.

The research described in this and subsequent papers was designed to study a number of the factors which might logically have some effect on the rate of deterioration of commercial preparations of these chemical types.

The ramifications of the problem made it necessary to divide it into parts and attack each part separately. Since this condition was due in a degree to the large number of both sodium hypochlorite and *p*-toluenesulfonchloramide preparations on the market it was decided to study first only the former. The latter substances would then be left until the completion of the initial phase of the problem. The effect of diffused daylight on the sodium hypochlorite solutions was the first factor considered. Under many conditions of use and storage, chlorine solutions are kept on open shelves without thought of protection from normal diffused daylight as it is found in the average room with two exposures. It is true that at times this light can become quite intense. However, its long-time effect without regard for the periods of high intensity was considered to be the factor of major importance. Since much has been said about the protective effect of colored glass this factor was examined also.

EXPERIMENTAL

Ten commercial preparations of chlorine which covered the field of use from those designed principally as antiseptics to those whose major utility is in the laundry were selected for study. They were purchased in the popular stock bottle sizes. Samples of each were then transferred to clear, blue, green and amber bottles of equivalent size. These were stored on open shelves in a laboratory having window exposure on the east and south. The shades were never drawn. The room temperature was fairly constant throughout the examination because of air conditioning. The number of sunny and cloudy days during the period of examination is shown in Table I.

The determinations of available chlorine were made approximately every 30 days for a series of ten analyses. The initial determination on each specimen was made at the time of transfer from the original or commercial container to the experimental containers. The U. S. P. XI method for the assay of solutions of sodium hypochlorite was used. This procedure was modified to a small degree in

* From the College of Pharmacy, University of Washington, Seattle, Wash.

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that 1-cc. samples were taken for the second to fifth determinations inclusive, and 5-cc. samples, accurately measured at 20° C., for the remainder. Also sulfuric acid was used in place of acetic acid, because the former gave more consistent results. This was demonstrated by a series of control assays in which the efficiency of both acids was carefully observed. The results of the determinations showing the rates of deterioration for the ten chlorine solutions studied under the conditions of the investigation are shown in Tables II to XI, inclusive. Because the majority of the manufacturers whose preparations were studied requested that their products be reported by number instead of name, each preparation will be known by the prefix C, with the appropriate serial number following. These designations will be carried throughout the study.

TABLE I.—NUMBER OF CLEAR, PARTLY CLOUDY AND CLOUDY DAYS DURING THE PERIOD OF INVESTIGATION (DATA TAKEN FROM THE OFFICIAL WEATHER REPORTS OF THE UNIVERSITY OF WASHINGTON DEPARTMENT OF GEOLOGY)

Month	Weather* No. of Clear Days	No. of Partly Cloudy Days	No. of Cloudy Days
May 24–31	4	1	2
June	18	7	5
July	15	7	9
August	17	3	11
September	13	6	11
October	2	5	24
November	1	3	27
December	8	1	22
January	7	3	21
February	9	2	17
March 1–20	11	3	6

* Average light intensity in laboratory: clear days, 50 candle power per sq. ft.; partly cloudy days, 33.5; cloudy days, 22.5.

TABLE II.—RATE OF DETERIORATION OF C-1 UNDER THE CONDITIONS OF THE STUDY

Period of Exposure in Days	Per Cent of Available Chlorine for Various Colors of Storage Bottles Clear	Green	Blue	Amber
0	1.01	0.98	0.99	1.01
28	0.86	0.91	0.90	0.97
62	0.72	0.83	0.74	0.98
94	0.51	0.65	0.58	0.87
127	0.44	0.68	0.59	0.96
160	0.40	0.65	0.52	0.99
188	0.37	0.62	0.47	0.97
234	0.35	0.58	0.39	0.96
256	0.32	0.55	0.33	0.95
275	0.28	0.50	0.32	0.94

TABLE III.—RATE OF DETERIORATION OF C-2 UNDER THE CONDITIONS OF THE STUDY

Period of Exposure in Days	Per Cent of Available Chlorine for Various Colors of Storage Bottles Clear	Green	Blue	Amber
0	4.66	4.65	4.65	4.59
28	4.12	4.48	4.38	4.52
62	3.63	4.09	4.04	4.46
94	2.96	...	...	...
127	2.92	3.78	3.54	4.36
160	2.55	3.52	3.20	4.28
188	2.38	3.45	3.04	4.16
234	2.30	3.31	2.95	4.12
256	2.16	3.18	2.78	3.92
275	1.96	3.09	2.72	3.93

TABLE IV.—RATE OF DETERIORATION OF C-3 UNDER THE CONDITIONS OF THE STUDY

Period of Exposure in Days	Per Cent of Available Chlorine for Various Colors of Storage Bottles Clear	Green	Blue	Amber
0	5.05	4.61	4.68	4.30
28	4.25	4.36	4.33	4.49
62	3.65	4.00	3.95	4.28
94	2.97	...	...	...
127	2.89	3.72	3.49	4.17
160	2.41	3.47	...	4.05
188	2.39	3.40	3.00	4.02
234	2.21	3.21	2.93	3.85
256	2.10	3.08	3.49	3.82
275	1.89	3.08	2.68	3.80

TABLE V.—RATE OF DETERIORATION OF C-4 UNDER THE CONDITIONS OF THE STUDY

Period of Exposure in Days	Per Cent of Available Chlorine for Various Colors of Storage Bottles Clear	Green	Blue	Amber
0	3.40	3.32	3.34	3.43
28	3.01	3.11	3.13	3.32
62	2.58	2.84	2.80	3.19
94	2.03	...	...	...
127	2.01	2.63	2.40	3.19
160	1.71	2.44	2.18	3.17
188	1.61	2.36	2.00	...
234	1.44	2.28	1.83	2.86
256	1.28	2.86	0.23	2.61
275	1.17	2.01	0.04	2.58

TABLE VI.—RATE OF DETERIORATION OF C-5 UNDER THE CONDITIONS OF THE STUDY

Period of Exposure in Days	Per Cent of Available Chlorine for Various Colors of Storage Bottles Clear	Green	Blue	Amber
0	4.73	4.66	4.69	4.80
28	4.17	4.44	3.94	4.52
62	4.15	3.91	3.93	4.41
94	2.85	3.48	3.36	...
127	2.88	...	3.16	4.25
160	2.55	3.45	...	4.26
188	2.45	3.36	3.03	4.15
234	2.12	3.22	2.81	4.03
256	2.06	3.05	2.58	...
275	2.03	3.00	2.56	3.94

TABLE VII.—RATE OF DETERIORATION OF C-6 UNDER THE CONDITIONS OF THE STUDY

Period of Exposure in Days	Per Cent of Available Chlorine for Various Colors of Storage Bottles Clear	Green	Amber
0	6.31	5.92	6.19
28	5.31	5.37	5.56
62	4.40	4.91	5.08
94	3.41	...	...
127	3.08	4.15	4.62
160	2.77	3.85	4.36
188	2.61	3.73	4.01
234	2.45	3.33	4.06
256	2.23	3.15	...
275	2.10	3.10	3.62

TABLE VIII.—RATE OF DETERIORATION OF C-7 UNDER THE CONDITIONS OF THE STUDY

Period of Exposure in Days	Per Cent of Available Chlorine for Various Colors of Storage Bottles			
	Clear	Green	Blue	Amber
0	2.48	2.47	2.25	2.62
28	2.16	2.30	2.39	2.46
62	1.67	2.09	2.19	2.39
94	1.10	1.71	1.73	2.32
127	0.90	1.79	1.04	...
160	0.61	0.67	0.24	2.37
188	0.26	0.12	0.02	2.35
234	0.05	0.04	0.01	1.67
256	0.04	0.03	0.01	0.04
275	0.03	0.03	0.01	0.04

TABLE IX.—RATE OF DETERIORATION OF C-8 UNDER THE CONDITIONS OF THE STUDY

Period of Exposure in Days	Per Cent of Available Chlorine for Various Colors of Storage Bottles			
	Clear	Green	Blue	Amber
0	4.13	4.18	4.15	4.19
28	3.64	3.80	3.70	3.97
62	2.97	3.39	3.09	3.70
94	2.14	2.68	2.24	3.21
127	1.90	2.84	2.05	3.22
160	1.57	2.07	1.62	2.86
188	1.38	1.78	1.40	2.56
234	1.14	1.40	1.13	2.05
256	0.99	1.22	0.97	1.88
275	0.88	1.13	0.90	1.77

TABLE X.—RATE OF DETERIORATION OF C-9 UNDER THE CONDITIONS OF THE STUDY

Period of Exposure in Days	Per Cent of Available Chlorine for Various Colors of Storage Bottles			
	Clear	Green	Blue	Amber
0	4.73	4.76	4.77	4.73
28	4.37	4.46	4.42	4.58
62	3.77	4.22	4.08	4.51
94	3.11	...	...	...
127	3.05	3.88	3.69	4.35
160	2.62	3.71	3.05	4.29
188	2.50	3.58	3.17	4.17
234	2.40	3.42	3.04	3.85
256	2.23	2.87	2.81	4.00
275	2.14	2.87	2.81	3.90

TABLE XI.—RATE OF DETERIORATION OF C-10 UNDER THE CONDITIONS OF THE STUDY

Period of Exposure in Days	Per Cent of Available Chlorine for Various Colors of Storage Bottles			
	Clear	Green	Blue	Amber
0	4.72	4.57	4.59	4.72
28	4.30	4.48	4.34	4.51
62	3.79	4.13	3.98	4.41
94	3.01	...	...	...
127	2.99	3.76	3.50	4.29
160	2.51	3.49	2.84	4.27
188	2.48	3.41	3.09	4.08
234	2.31	3.27	2.93	4.01
256	2.15	3.07	2.75	3.73
275	1.96	3.04	2.70	3.69

DISCUSSION

The data collected are summarized in Tables XII, XIII and XIV. From these tables, it is evident that amber glass containers offer better protection against the destructive effect of diffused daylight than the other bottles used. In order of effective protection value the four containers rate: (1) amber, (2) blue, (3) green and (4) clear glass. It is also apparent that if the majority of the preparations studied are used within a month or so after purchase their approximate maximum utility is available.

TABLE XII.—A COMPARISON OF THE DEGREE OF TOTAL DETERIORATION OF THE SAMPLES STUDIED

Preparation	Per Cent Total Deterioration during Study			
	Clear Bottles	Green Bottles	Blue Bottles	Amber Bottles
C-1	62.38	48.98	67.67	6.93
C-2	58.58	33.54	41.50	14.38
C-3	62.57	33.18	42.73	11.62
C-4	65.58	39.45	98.50	24.78
C-5	57.08	35.62	45.42	17.91
C-6	66.72	47.63	...	41.52
C-7	98.79	98.78	99.55	98.48
C-8	78.69	72.97	78.31	57.75
C-9	54.75	39.70	41.09	18.19
C-10	58.48	33.48	41.18	21.82

TABLE XIII.—ORDER OF DETERIORATION

The products are listed under each container according to their degree of deterioration. The one showing the least deterioration is listed first, and so on.

Clear Bottles	Green Bottles	Blue Bottles	Amber Bottles
C-9	C-3	C-9	C-1
C-5	C-10	C-10	C-3
C-10	C-2	C-2	C-2
C-2	C-5	C-3	C-5
C-1	C-4	C-5	C-9
C-3	C-9	C-1	C-10
C-4	C-6	C-8	C-4
C-6	C-1	C-4	C-6
C-8	C-8	C-7	C-8
C-7	C-7	...	C-7

TABLE XIV.—DETERIORATION RATINGS

The number assigned each product indicates its position according to degree of deterioration in the different containers. Its deterioration rating is obtained by adding all the position numbers and dividing by the number of positions, which is 4.

Preparation	Position in				Total Position Points	Average Position Points
	Clear Glass	Green Glass	Blue Glass	Amber Glass		
C-9	1	6	1	5	13	3.25
C-5	2	4	5	4	15	3.75
C-10	3	2	2	6	13	3.25
C-2	4	3	3	3	13	3.25
C-1	5	8	6	1	20	5.00
C-5	6	1	4	2	13	3.25
C-4	7	5	8	7	27	6.75
C-6	8	7	..	8	23[a]	7.66[a]
C-8	9	9	7	9	34	8.50
C-7	10	10	9	10	39	9.75

[a] Based on three ratings only.

Storage Factors Affecting the Deterioration Rates of Certain Chlorine Preparations of the Sodium Hypochlorite Type*

II. The Effect of Direct Sunlight

By Elmer M. Plein and L. Wait Rising

Although it is not customary to store chlorine preparations of the type investigated in this series of experiments where they will be exposed all day long to the direct rays of the sun, concrete knowledge concerning the hazard of the condition should nevertheless be made available. There are people who without thought of conservation of efficiency set such products in windows and other exposed places. What in specific terms are they doing to the strength of their chlorine preparations?

EXPERIMENTAL

To answer this question and also to determine the effectiveness of various colors of glass as protection against the action of light, the ten chlorine preparations examined in the first study of the series (1) were removed from their stock bottles and placed in clear, green and blue glass pint bottles. As the products were originally packaged in amber bottles by their manufacturers these containers were used as the amber bottles. These sets were then stored on the roof of the laboratory building where they were exposed to whatever sunlight was available. The weather conditions, together with the mean temperatures for each month of exposure, are shown in Table I. It is important to note that the maximum sunlight was impinging upon the bottles from the very beginning of the investigation until after the effect of sunlight was no longer so significant.

The preparations were analyzed in accordance with the procedure employed in study I (1). The data collected are given in Tables II to XI, inclusive. Table XII compares the degree of deterioration of each for the period of the study.

TABLE I.—NUMBER OF CLEAR, PARTLY CLOUDY AND CLOUDY DAYS DURING THE PERIOD OF INVESTIGATION (DATA TAKEN FROM THE OFFICIAL WEATHER REPORTS OF THE UNIVERSITY OF WASHINGTON DEPARTMENT OF GEOLOGY)

Month	Weather: No. of Clear Days	No. of Partly Cloudy Days	No. of Cloudy Days	Mean Temperatures, °F.
May 24–31	4	1	2	58.79
June	18	7	5	63.43
July	15	7	9	64.90
August	17	3	11	64.30
September	13	6	11	54.40
October	2	5	24	56.35
November	1	3	27	43.58
December	8	1	22	44.28
January	7	3	21	43.63
February	9	2	17	46.32
March 1–20	11	3	6	51.12

TABLE II.—RATE OF DETERIORATION OF C-5 UNDER THE CONDITIONS OF THE STUDY

Date of Chlorine Determination	Per Cent of Available Chlorine for Various Colors of Storage Bottles: Clear	Green	Blue	Amber
May 24, 1940 (Initial)	4.25	(Bottle broken)	4.49	...
June 15, 1940	0.00	...	0.00	4.60
July 1, 1940	0.01	...	0.00	4.12
July 15, 1940	0.00	...	...	3.73
Aug. 15, 1940	...	...	...	3.23
Sept. 18, 1940	...	...	...	...
Oct. 17, 1940	...	...	...	2.77
Nov. 1, 1940	...	4.18[a]	...	...
Nov. 15, 1940	...	...	...	2.71
Nov. 29, 1940	...	1.96	...	...
Dec. 18, 1940	...	0.05	...	2.68
Jan. 15, 1941	...	...	...	2.63
Feb. 23, 1941	...	...	...	2.42
Apr. 2, 1941	...	...	...	2.25

[a] A new series of determinations begun.

TABLE III.—RATE OF DETERIORATION OF C-7 UNDER THE CONDITIONS OF THE STUDY

Date of Chlorine Determination	Per Cent of Available Chlorine for Various Colors of Storage Bottles: Clear	Green	Blue	Amber
May 24, 1940 (Initial)	2.66	2.69	2.77	2.68
June 15, 1940	0.01	0.01	0.01	2.41
July 1, 1940	0.01	0.01	0.01	2.10
July 15, 1940	...	...	...	2.02
Aug. 15, 1940	...	0.01	0.01	1.79
Sept. 18, 1940	...	0.01	...	...
Oct. 17, 1940	...	...	...	1.59
Nov. 1, 1940	1.30[a]	...	...	...
Nov. 15, 1940	...	...	...	1.42
Nov. 29, 1940	0.01	...	...	...
Dec. 18, 1940	...	...	...	1.34
Jan. 15, 1941	...	...	...	1.06
Feb. 24, 1941	...	...	...	0.32
Apr. 2, 1941	...	...	...	0.24

[a] A new series of determinations begun.

* From the College of Pharmacy, University of Washington, Seattle, Wash.

Presented to the Scientific Section of the A. PH. A., Detroit meeting, 1941.

TABLE IV.—RATE OF DETERIORATION OF C-6 UNDER THE CONDITIONS OF THE STUDY

Date of Chlorine Determination	Per Cent of Available Chlorine for Various Colors of Storage Bottles: Clear	Green	Blue	Amber
May 24, 1940 (Initial)	5.13	5.20	5.00	...
June 15, 1940	...	0.41	0.32	5.22
July 1, 1940	0.02	0.01	0.01	3.46
July 15, 1940	...	0.01	0.01	2.67
Aug. 15, 1940	0.01	0.01	0.01	1.54
Sept. 18, 1940	0.01	0.01	0.01	0.98
Oct. 17, 1940	0.01	0.01	0.01	0.88
Nov. 15, 1940	...	...	...	0.79
Dec. 18, 1940	...	...	...	0.75
Jan. 15, 1941	...	...	...	0.74
Feb. 24, 1941	...	...	...	0.64
Apr. 2, 1941	...	...	...	0.52

TABLE V.—RATE OF DETERIORATION OF C-8 UNDER THE CONDITIONS OF THE STUDY

Date of Chlorine Determination	Per Cent of Available Chlorine for Various Colors of Storage Bottles: Clear	Green	Blue	Amber
May 24, 1940 (Initial)	4.45	4.68	4.48	4.45
June 15, 1940	0.01	0.00	0.00	4.32
July 1, 1940	0.01	...	...	3.79
July 15, 1940	0.00	...	...	3.55
Aug. 15, 1940	...	...	...	3.06
Sept. 18, 1940	...	...	...	...
Oct. 17, 1940	...	...	...	2.46
Nov. 15, 1940	...	...	...	2.30
Dec. 18, 1940	...	...	...	2.27
Jan. 15, 1941	...	...	...	2.17
Feb. 24, 1941	...	...	...	2.06
Apr. 2, 1941	...	...	...	1.84

TABLE VI.—RATE OF DETERIORATION OF C-9 UNDER THE CONDITIONS OF THE STUDY

Date of Chlorine Determination	Per Cent of Available Chlorine for Various Colors of Storage Bottles: Clear	Green	Blue	Amber
May 24, 1940 (Initial)	5.07	5.12	4.70	4.72
June 15, 1940	0.00	0.01	0.00	4.72
July 1, 1940	...	0.00	...	4.45
July 15, 1940	...	...	...	4.30
Aug. 15, 1940	...	...	...	4.04
Sept. 18, 1940	...	...	...	3.89
Oct. 17, 1940	...	...	...	3.75
Nov. 15, 1940	...	...	...	3.75
Dec. 18, 1940	...	...	...	3.73
Jan. 15, 1941	...	...	...	3.62
Feb. 24, 1941	...	...	...	3.48
Apr. 2, 1941	...	...	...	3.48

TABLE VII.—RATE OF DETERIORATION OF C-10 UNDER THE CONDITIONS OF THE STUDY

Date of Chlorine Determination	Per Cent of Available Chlorine for Various Colors of Storage Bottles: Clear	Green	Blue	Amber
May 24, 1940 (Initial)	4.71	4.67	...	4.79
June 15, 1940	0.01	0.03	...	4.67
July 1, 1940	0.01	0.01	...	4.19
July 15, 1940	0.00	0.01	...	4.06
Aug. 15, 1940	...	0.00	...	3.71
Sept. 18, 1940	...	...	...	3.48
Oct. 17, 1940	...	...	...	3.41
Nov. 1, 1940	...	...	4.35[a]	...
Nov. 15, 1940	...	...	...	...
Nov. 29, 1940	...	...	1.24	...
Dec. 18, 1940	...	...	0.05	3.19
Jan. 15, 1941	...	...	0.00	...
Feb. 24, 1941	...	...	...	2.88
Apr. 2, 1941	...	...	...	2.93

[a] Initial determination.

(Table VII continued in next column)

TABLE VIII.—RATE OF DETERIORATION OF C-2 UNDER THE CONDITIONS OF THE STUDY

Date of Chlorine Determination	Per Cent of Available Chlorine for Various Colors of Storage Bottles: Clear	Green	Blue	Amber
May 24, 1940 (Initial)	4.50	...	4.57	4.87
June 15, 1940	0.01	...	0.01	4.72
July 1, 1940	0.01	...	0.01	4.37
July 15, 1940	0.00	...	0.00	4.27
Aug. 15, 1940	...	...	...	3.86
Sept. 18, 1940	...	...	...	...
Oct. 17, 1940	...	...	...	...
Nov. 1, 1940	...	4.27[a]	...	...
Nov. 15, 1940	...	...	...	3.83
Dec. 18, 1940	...	1.10	...	3.71
Jan. 15, 1941	...	0.00	...	...
Feb. 24, 1941	...	...	...	3.43
Apr. 2, 1941	...	...	...	3.46

[a] Initial determination.

TABLE IX.—RATE OF DETERIORATION OF C-3 UNDER THE CONDITIONS OF THE STUDY

Date of Chlorine Determination	Per Cent of Available Chlorine for Various Colors of Storage Bottles: Clear	Green	Blue	Amber
May 24, 1940 (Initial)	4.72	4.75	4.84	4.89
June 15, 1940	0.00	0.00	0.01	4.10
July 1, 1940	...	...	0.00	2.94
July 15, 1940	...	...	...	2.31
Aug. 15, 1940	...	...	...	1.41
Sept. 18, 1940	...	...	...	0.88
Oct. 17, 1940	...	...	...	0.81
Nov. 15, 1940	...	...	...	0.73
Dec. 18, 1940	...	...	...	0.69
Jan. 15, 1941	...	...	...	0.68
Feb. 24, 1941	...	...	...	0.57
Apr. 2, 1941	...	...	...	0.46

TABLE X.—RATE OF DETERIORATION OF C-4 UNDER THE CONDITIONS OF THE STUDY

Date of Chlorine Determination	Per Cent of Available Chlorine for Various Colors of Storage Bottles: Clear	Green	Blue	Amber
May 24, 1940 (Initial)	2.98	3.58	3.58	3.55
June 15, 1940	0.00	0.00	0.00	3.34
July 1, 1940	...	...	...	2.98
July 15, 1940	...	...	...	2.67
Aug. 15, 1940	...	...	...	2.41
Sept. 18, 1940	...	...	...	...
Oct. 17, 1940	...	...	...	2.13
Nov. 15, 1940	...	...	...	1.94
Dec. 18, 1940	...	...	...	1.70
Jan. 15, 1941	...	...	...	0.45
Feb. 24, 1941	...	...	...	0.07
Apr. 2, 1941	...	...	...	0.04

TABLE XI.—RATE OF DETERIORATION OF C-1 UNDER THE CONDITIONS OF THE STUDY

Date of Chlorine Determination	Per Cent of Available Chlorine for Various Colors of Storage Bottles: Clear	Green	Blue	Amber
May 24, 1940 (Initial)	0.96	1.01	1.01	1.06
June 15, 1940	0.00	0.00	0.00	0.98
July 1, 1940	...	...	...	0.94
July 15, 1940	...	...	...	0.89
Aug. 15, 1940	...	...	...	0.85
Sept. 18, 1940	...	...	...	...
Oct. 17, 1940	...	...	...	0.82
Nov. 15, 1940	...	...	...	0.78
Dec. 18, 1940	...	...	...	0.78
Jan. 15, 1941	...	...	...	0.72
Feb. 23, 1941	...	...	...	0.70
Apr. 2, 1941	...	...	...	0.67

TABLE XII.—A COMPARISON OF THE DEGREE OF TOTAL DETERIORATION OF SAMPLES STUDIED

Preparation	Per Cent Total Deterioration during Study: Clear Bottles	Green Bottles	Blue Bottles	Amber Bottles
C-5	100.00	98.80	100.00	46.68
C-7	99.63	100.00	99.64	91.05
C-6	99.81	99.81	99.80	90.04
C-8	100.00	100.00	100.00	58.65
C-9	100.00	100.00	100.00	31.02
C-10	100.00	100.00	98.85	38.83
C-2	100.00	74.24[a]	100.00	28.95
C-3	100.00	100.00	100.00	90.59
C-4	100.00	100.00	100.00	98.87
C-1	100.00	100.00	100.00	36.79

[a] Forty-eight days.

TABLE XIII.—COMPARISON OF DEGREE OF TOTAL DETERIORATION OF THE SAMPLES IN AMBER BOTTLES UNDER THE INFLUENCE OF DIRECT SUNLIGHT AND DIFFUSED SUNLIGHT

Preparation	Per Cent Deterioration under Direct Sunlight	Per Cent Deterioration under Diffused Sunlight
C-2	28.95	14.38
C-9	31.02	18.19
C-1	36.79	6.93
C-10	38.83	21.82
C-5	46.68	17.91
C-8	58.65	57.75
C-6	90.04	41.52
C-3	90.59	11.62
C-7	91.05	98.48
C-4	98.87	24.78

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(1) Plein, E. M., and Rising, L. W., JOUR. A PH. A., 31 (1942), 183.

Storage Factors Affecting the Deterioration Rates of Certain Chlorine Preparations of the Sodium Hypochlorite Type*

III. (*A*) The Effect of Constant Moderate Heat, (*B*) The Effect of Storage in a Dark Room and (*C*) The Effect of Refrigeration

By Elmer M. Plein and L. Wait Rising

(*A*) The Effect of Constant Moderate Heat

Many of the chlorine preparations used routinely in the home for hygienic purposes on the person and in the laundry, also those used in hotels, taverns, hospitals and elsewhere to protect the public against the spread of contagious diseases, are stored in warmer-than-average places. This is not intentional. It is pure coincidence that so frequently the out-of-the-way corners and cabinets convenient for storage are near steam pipes, heating units or hot water pipes. That this heat can be damaging to the activity of the chlorine preparations no one can successfully deny without previously having measured its effect. Published accounts showing adequate measurements of its effect are lacking, so the work reported in this paper was undertaken to help answer the question on the basis of data collected under controlled conditions.

* From the College of Pharmacy, University of Washington, Seattle, Wash.

Presented to the Scientific Section of the A. PH. A., Detroit meeting, 1941.

EXPERIMENTAL

Standard commercial packages of the ten preparations used in the previous phases (1) of this study were used. Since light was not to be considered as a deterioration factor, the products were left in their original containers. These were stored in a covered box placed over a copper chest in which small steam pipes were coiled. The temperature of the liquids in the box, without considering the few fluctuations when the steam was cut off, averaged about 42° C. The samples were analyzed according to the time intervals and method of the previous studies (1). Table I shows the results of the determinations. Table II shows the total deterioration of each over the period of investigation.

TABLE I.—RATE OF DETERIORATION OF THE CHLORINE PREPARATIONS UNDER THE CONDITIONS OF THE STUDY

Date of Chlorine Determination	Per Cent of Available Chlorine in Preparations									
	C-5	C-1	C-4	C-3	C-2	C-10	C-9	C-8	C-6	C-7
May 23, 1940 (Initial)	4.81	1.02	3.56	4.81	4.86	4.88	5.02	4.27	5.13	2.62
June 19, 1940	4.35	0.94	3.19	4.34	4.29	4.34	4.34	3.80	4.52	2.32
July 15, 1940	3.52	0.90	2.91	3.50	3.42	3.50	3.73	1.94	3.13	1.88
Aug. 16, 1940	2.96	0.86	2.50	3.14	2.75	1.97	2.97	1.10	2.94	1.31
Sept. 19, 1940	2.23	0.72	1.87	2.39	2.06	1.92	2.49	0.68	2.67	0.94
Oct, 22, 1940	2.26	0.71	1.82	2.41	1.97	1.83	2.10	0.59	1.30	0.95
Nov. 18, 1940	2.10	0.76	1.83	2.40	1.96	1.85	2.19	0.61	1.15	0.88
Dec. 23, 1940	1.94	0.72	1.69	2.22	1.83	1.71	1.91	0.54	0.93	0.79
Feb. 12, 1941	1.70	0.65	1.48	1.85	1.49	1.41	1.63	0.44	0.75	0.65
Feb. 28, 1941	1.64	0.55	1.44	1.75	1.43	1.30	1.55	0.41	0.67	0.60
Apr. 2, 1941	1.57	0.55	1.35	1.64	1.33	1.23	1.42	0.47	0.60	0.19

TABLE II.—A COMPARISON OF THE DEGREE OF TOTAL DETERIORATION OF THE SAMPLES STUDIED

Preparation	Per Cent Total Deterioration during Study
C-1	46.08
C-4	62.08
C-3	64.66
C-5	67.36
C-9	71.71
C-2	72.63
C-10	74.79
C-6	88.30
C-8	89.99
C-7	92.75

(*B*) The Effect of Storage in a Dark Room

In previous phases of this study (2) it was found that the direct sunlight had a most deleterious effect on the sodium hypochlorite preparations being investigated, even when protected by amber bottles. Diffused daylight (1) was also destructive, but not to the same degree. Amber bottles offered a great deal more protection against the ravages of diffused daylight than against direct sunlight. Since decreased light intensity resulted in greatly decreased deterioration, we tried the protective effect of total darkness broken only by intermittent light from electric light bulbs such as would be found in inside storage rooms and dark basements.

EXPERIMENTAL

The ten preparations under investigation were stored in a dark room in their original amber glass containers. They were exposed intermittently, as previously stated, to electric light rays. The temperature of the room was reasonably constant and never warm. Analyses were made in the usual way (1) and the results tabulated in Tables III and IV. From the data collected it will be seen that while the absence of light greatly reduces deterioration, it does not inhibit it completely.

TABLE IV.—A COMPARISON OF THE DEGREE OF TOTAL DETERIORATION OF THE SAMPLES STUDIED

Preparation	Per Cent Total Deterioration during Study
C-4	10.43
C-2	17.20
C-7	17.36
C-5	17.38
C-3	17.99
C-10	19.51
C-9	23.83
C-1	30.39
C-6	33.21
C-8	64.83

TABLE III.—RATE OF DETERIORATION OF THE CHLORINE PREPARATIONS UNDER THE CONDITIONS OF THE STUDY

Date of Chlorine Determination	Per Cent of Available Chlorine in Preparations									
	C-5	C-7	C-4	C-3	C-2	C-10	C-9	C-8	C-6	C-7
May 28, 1940 (Initial)	4.89	1.00	3.45	4.78	4.67	4.92	4.91	4.35	5.42	2.65
July 16, 1940	4.60	0.96	3.27	4.63	4.46	4.62	4.57	4.08	...	2.44
Aug. 16, 1940	4.38	0.95	3.26	4.41	4.37	4.36	4.42	3.76	...	2.38
Oct. 18, 1940	4.27	...	...	4.35	...	...	4.32	3.28	4.44	...
Nov. 22, 1940	...	...	...	4.21	4.20	4.18	4.25	2.74	...	2.37
Dec. 19, 1940	4.12	...	3.14	4.13	4.12	4.03	...	2.40	4.29	2.37
Jan. 18, 1941	...	0.91	3.12	...	3.93	3.98	4.17	2.16	4.27	2.36
Feb. 21, 1941	4.10	0.86	3.06	3.98	...	...	3.97	1.83	3.62	2.31
Mar. 15, 1941	4.04	0.71	2.99	3.92	3.90	3.96	3.74	1.53	2.62	2.19

(C) The Effect of Refrigeration

Continuing our study of the factors influencing the deterioration of the commercially available sodium hypochlorite type chlorine preparations, we next investigated the effect of low temperatures on their stability. Low temperatures are considered stabilizers for most types of perishable chemicals and particularly so for those which are extremely unstable. It was presumed, therefore, that refrigeration would probably inhibit deterioration to a large degree, if not prevent it completely in our preparations. The work to be done, therefore, was looked upon as merely confirmatory.

EXPERIMENTAL

Samples of the ten preparations being investigated were refrigerated in an electric ice box of standard make and at the temperature usually used for food preservation in the home. The solutions were removed only long enough during each analysis to come to the standard temperature to be pipetted. The pipetted samples were then titrated with 0.1 *N* sodium thiosulfate in the usual way (1). Some of the data collected are shown in Table V. The solutions are identified in the first column. The original strengths of the preparations are given in the second column, and the final strengths, 260 days later, in the third column. In the last column are reported the percentage deterioration of each solution. The percentage deterioration ranged from 1.06 to 22.03.

TABLE V.—PER CENT STRENGTHS OF THE SAMPLES UNDER THE CONDITIONS OF THE STUDY AND THEIR PER CENT DETERIORATIONS

Preparation	Initial Strength, Per Cent Available Chlorine, May 28, 1940	Final Strength, Per Cent Available Chlorine, Feb. 12, 1941	Per Cent Deterioration
C-9	4.71	4.66	1.06
C-7	2.64	2.59	1.89
C-3	4.76	4.64	2.52
C-4	3.52	3.43	2.56
C-1	1.00	0.97	3.00
C-2	4.60	4.31	6.30
C-8	4.55	3.90	14.29
C-5	4.73	3.97	16.07
C-6	5.77	4.72	18.20
C-10	4.72	3.68	22.03

There seems to be no correlation of the deterioration rates of the chlorine solutions under the various conditions of storage. For example, solution C-1 is the most stable of the solutions when stored under constant moderate heat (Table II), but this solution ranks eighth when stored in a dark room (Table IV) and fifth when held under refrigeration (Table V).

REFERENCES

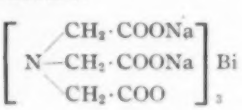
(1) Plein, E. M., and Rising, L. W., JOUR. A. PH. A., 31 (1942), 183.

(2) Plein, E. M. and Rising, L. W., *Ibid.*, 31 (1942), 186.

Sodium Bismuth Triglycollamate*

By Robert A. Lehman and Reavis C. Sproull

In 1925 Hahl (1) disclosed a method for preparing a compound to which he gave the following structure:



(I)

Hahl's procedure consisted in warming together sodium carbonate, triglycollamic acid (*sym*-tricarboxytrimethylamine) and bismuth oxide until all were dissolved, and then evaporating to dryness. However, the quantities of reagents given do not agree even approximately with the structure suggested. Furthermore, Michaelis and Schubert (2) have shown by potentiometric titration of triglycollamic acid that two of the carboxyl groups are highly ionized (*p*K 3) and indistinguishable, while the third carboxyl group exists as a "zwitterion" (*p*K 10). A solution of disodium triglycollamate is thus nearly neutral (*p*H 8), while the trisodium salt is quite alkaline (*p*H 11). In view of these properties of the acid, it seems improbable that a compound of structure (I) would give a stable and neutral aqueous solution as claimed by Hahl.

Preliminary experiments showed that sodium bismuth triglycollamate possesses pharmacological and chemical properties which, in certain respects, differentiate it

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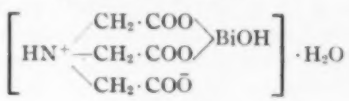
from other soluble bismuth compounds used in medicine. It therefore appeared desirable to reinvestigate the chemistry of the compound.

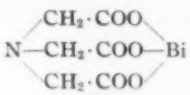
DISCUSSION AND RESULTS

When a hot aqueous solution of triglycollamic acid is boiled with one mol equivalent of bismuth (as Bi_2O_3), a clear solution results, which, upon cooling, throws down, in good yield, crystals whose structure may best be formulated as follows:



(II)

This substance can be readily recrystallized from water without hydrolysis of the bismuth. On drying to constant weight (II) loses two molecules of water probably giving (III):



(III)

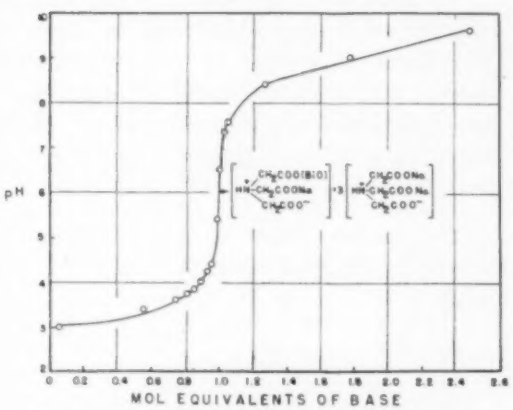
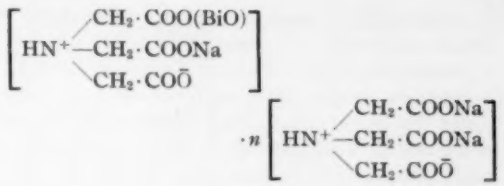


Fig. 1.—Titration of acid bismuth triglycollamate (II) in the presence of three mol equivalents of disodium triglycollamate.

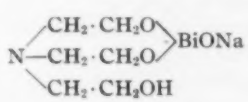
A saturated aqueous solution of (II) is quite unstable with respect to chemical reagents, for hydroxyl, chloride or phosphate ions precipitate the appropriate insoluble bismuth salts. While only slightly soluble in cold water, (II) is very soluble in a solution of disodium triglycollamate, presumably forming a series of double salts or complex ions. A 0.05 *M* solution of (II) containing three mol equivalents of disodium triglycollamate has a *p*H of 3.0 and gives the titration curve shown in Fig. 1. It will be seen that one mol equivalent of base has been consumed at the inflection, at which point the solution is substantially neutral. Neutralization of one carboxyl group in (II) with sodium hydroxide in the presence of excess disodium triglycollamate might thus give solutions of the following series of compounds:



(IV)

Moreover, the presence of the disodium triglycollamate greatly increases the stability of these solutions toward chemical reagents and in proportion to the amount present. Thus, when n is 3, and the resulting solution is 0.05 *M* with respect to bismuth, no precipitation occurs upon addition of phosphate or chloride ions, nor within the *p*H range 2.8 to 10.0. Such a solution is stable to light and when sterile can be stored indefinitely. It can be evaporated to dryness or greatly diluted without chemical change. A crystalline double salt corresponding to structure (IV) where n is 1 has been prepared and found to be less stable than when n is 2 or 3, but decidedly more stable than the sodium salt of (II). While nothing can be said as to the nature of the complexes whose formation is postulated, nevertheless their solutions are rather unique in the wide variation in conditions under which they show no chemical or physical change. The pharmacology of these compounds will be reported elsewhere; it may be mentioned, however, that they are of sufficiently low toxicity to make their therapeutic application feasible.

Recently Miller (3) has prepared unstable bismuth derivatives of triethanolamine and triisopropanolamine which he believed to be similar to the active principles present in Hanzlik's empirical mixture, Sobisminol (4). The structure advanced by Miller for one of these compounds is:



(V)

It has been pointed out that the initial reaction in the preparation of Sobisminol is a reduction of the sodium bismuthate. If this takes place at the expense of the triisopropanolamine, compounds of structures similar to (II) might possibly be obtained. However, the quantities of reactants used by Hanzlik would only account for the conversion of a portion of the bismuthate to a substance of this kind.

EXPERIMENTAL

Preparation of Triglycollamic Acid.—This was prepared as described by Michaelis and Schubert (2) except that the much less expensive chloracetic instead of iodoacetic acid was used. Yield, 60%; m. p. (with decomposition), 234° C. (uncorr.). Anal. Calcd. for $C_6H_9O_6N$: C, 37.7; H, 4.71. Found. C, 37.7; H, 4.35. The disodium salt was prepared

by dissolving the acid in two mol equivalents of sodium hydroxide and evaporating to dryness.

Preparation of Acid Bismuth Triglycollamate (II).—Fourteen grams (0.03 mol) of bismuth oxide (calcd. for Bi_2O_3: 89.7% Bi; found: 89.1%) were suspended in a solution of 11.5 Gm. (0.06 mol) of triglycollamic acid in four liters of boiling water. The suspension was boiled until a clear or only slightly turbid solution resulted. This required from 5 to 10 min. The solution was then filtered and chilled to 5° C. The fine, colorless needles were filtered off with suction, washed with ice water, alcohol and ether, and air dried. Yield, 20 Gm., 77%. The salt was recrystallized from water three times before analysis. Hydrolysis was prevented by dissolving the crystals directly in a just sufficient amount of distilled water, already at the boiling point, filtering and chilling rapidly. About 125 cc. of water is required per Gm. of salt. Solubility at room temperature is about 0.09 Gm. per 100 cc. The salt decomposes at 258–262° C. (uncorr.) when the temperature of the melting point bath is raised at the rate of 2 degrees per minute. Bismuth was determined by digestion with nitric, sulfuric and perchloric acids, double precipitation as basic carbonate and ignition to Bi_2O_3. The water content was determined by drying to constant weight at 125° C.

Anal. Calcd. for $C_6H_{10}O_8NBi$: Bi, 48.3; N, 3.24; H_2O, 8.31. Found: Bi, 48.2; N, 3.10; H_2O, 8.25.

A solution of acid bismuth triglycollamate (II) was prepared by dissolving 4.33 Gm. (0.01 mol) of (II) and 7.05 Gm. (0.03 mol) of disodium triglycollamate in water and diluting to 100 cc. This 0.1 *M* solution was then titrated with 0.1 *M* sodium hydroxide using the glass electrode, and the titration curve is shown in the figure.

The structure of (II) is presumed to follow from the method of preparation, the titration curve and the analytical data. Structure (III) is the only one that could account for the loss of two molecules of water from (II) without anhydride formation. Since triglycollamic acid itself does not form an anhydride nor lose weight at 125° C., (III) is believed to be correct.

Preparation of Double Salt (IV) $C_4H_7O_2N(COONa)$-$(COOBiO)$-$C_4H_7O_2N(COONa)_2$-$4H_2O$.—To 7.64 Gm. (0.04 mol) of triglycollamic acid suspended in about 100 cc. of water was added 3.18 Gm. (0.03 mol) of anhydrous sodium carbonate dissolved in 50 cc. of water. The resulting solution was brought to boiling and 4.66 Gm. (0.01 mol) of bismuth oxide was added in small portions. It is preferable to triturate the oxide with water beforehand. The mixture was then boiled until clear, filtered and evaporated on the steam bath to about 35-cc. volume. Upon standing over night at room temperature, large colorless prisms were formed which were collected on a filter and washed with small portions of ice water. The material was recrystallized from a small volume of hot water in which case several days were required for the crystals to form. After filtering and washing as before, the double salt was dried quickly between filter paper. It was then suspended in alcohol, filtered, washed on the filter with ether and briefly air dried. The crystals must be preserved in a sealed container since they effloresce rapidly in dry air. Yield, 40%. Anal. Calcd. for $C_{12}H_{22}O_{17}N_2BiNa_3$: Bi, 28.1; N, 3.76; H_2O, 9.68. Found: Bi, 28.3; N, 3.70; H_2O, 9.69.

SUMMARY

Methods have been given for the preparation of a well-crystallized acid bismuth triglycollamate and a hydrated crystalline double salt of sodium bismuth triglycollamate with disodium triglycollamate. Structures have been suggested and properties described.

The authors wish to express their appreciation for the assistance of Miss Theis Aitken.

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(3) Miller, W. T., *J. Am. Chem. Soc.*, 62 (1940), 2707.

(4) Report of the Council on Pharmacy and Chemistry, *J. Am. Med. Assoc.*, 113 (1939), 2214.